NOAA Technical Memorandum NMFS

FEBRUARY 2000

MOLECULAR GENETIC IDENTIFICATION OF WHALES, DOLPHINS, AND PORPOISES: PROCEEDINGS OF A WORKSHOP ON THE FORENSIC USE OF MOLECULAR TECHNIQUES TO IDENTIFY WILDLIFE PRODUCTS IN THE MARKETPLACE

Andrew Dizon
Scott Baker
Frank Cipriano
Gina Lento
Per Palsbøll
Randall Reeves

NOAA-TM-NMFS-SWFSC-286

U.S. DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration
National Marine Fisheries Service
Southwest Fisheries Science Center

The U.S. National Oceanic and Atmospheric Administration (NOAA)–organized in 1970–has evolved into an agency which establishes national policies, and manages and conserves U.S. oceanic, coastal, and atmospheric resources. An organizational element within NOAA–the Office of Fisheries–is responsible for fisheries policy and the direction of the National Marine Fisheries Service (NMFS).

In addition to its formal publications, the NMFS uses the NOAA Technical Memorandum series to issue informal scientific and technical publications when complete formal review and editorial processing are not appropriate or feasible. Documents within this series, however, reflect sound professional work, and may be referenced in the formal scientific and technical literature.

How to cite this report:

Dizon, A., C. S. Baker, F. Cipriano, G. Lento, P. Palsbøll, and R. Reeves. (eds.) 2000. Molecular Genetic Identification of Whales, Dolphins, and Porpoises: Proceedings of a Workshop on the Forensic Use of Molecular Techniques to Identify Wildlife Products in the Marketplace. La Jolla, CA, USA, 14-16 June 1999. U.S. Department of Commerce, NOAA Technical Memorandum, NOAA-TM-NMFS-SWFSC-286. 52pp. + xi.

NOAA Technical Memorandum NMFS
This TM series is used for documentation and timely communication of preliminary results, interim reports, or special purpose information. The TMs have not received complete formal review, editorial control, or detailed editing.

FEBRUARY 2000

MOLECULAR GENETIC IDENTIFICATION OF WHALES, DOLPHINS, AND PORPOISES: PROCEEDINGS OF A WORKSHOP ON THE FORENSIC USE OF MOLECULAR TECHNIQUES TO IDENTIFY WILDLIFE PRODUCTS IN THE MARKETPLACE

Andrew Dizon
Southwest Fisheries Science Center
National Marine Fisheries Service
La Jolla, California, USA

Scott Baker
School of Biological Sciences
Auckland University
Auckland, New Zealand

Frank Cipriano
Department of Biology
San Francisco State University
San Francisco, California USA

Gina Lento
School of Biological Sciences
Auckland University
Auckland, New Zealand

Per Parsbøll
School of Biological Sciences
University of Wales
Bangor, Wales, UK

Randall Reeves (Workshop Chair)
Okapi Wildlife Associates
Hudson, Quebec
Canada

NOAA-TM-NMFS-SWFSC-286

[This page intentionally left blank]

TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

LIST OF APPENDICES

EXECUTIVE SUMMARY

With the International Whaling Commission's (IWC's) moratorium on commercial whaling in effect for more than a decade, several research teams have sampled cetacean products in Asian markets and restaurants, particularly in Japan and Republic of Korea (South Korea), and used molecular genetic techniques[1] to identify which species and stocks are represented. This forensic use of molecular techniques to identify wildlife products in the marketplace is of practical interest to the IWC as it develops a Revised Management Scheme (RMS) to regulate any future whaling, and to the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), which is responsible for regulating the international trade in whale products.

A scientific workshop was held at the U.S. Government's Southwest Fisheries Science Center (SWFSC) in La Jolla, California, 14-16 June 1999. The workshop was sponsored by the SWFSC, the International Fund for Animal Welfare (IFAW) and the World Wildlife Fund (WWF), with invited participants from nine countries representing academic institutions, governmental research laboratories, and non-governmental organizations (NGOs) including TRAFFIC. The objectives of the workshop were to:

- Review and summarize the results of forensic studies of cetacean products in Asian markets since the international moratorium on commercial hunting of certain species took effect in 1986;
- Review the current scientific and technical methods for molecular identification of cetaceans, including consideration of progress made with other taxonomic groups;
- Identify and evaluate new analytical approaches that could be of use in the molecular identification of cetaceans;
- Review and evaluate methods used to sample product markets;
- Review the status of reference collections and databases and consider ongoing needs to procure and gain access to genetic information.

About 70% of the cetacean products from market surveys in Japan and South Korea have proven to be from minke whales (about 19% Northern Hemisphere *[Balaenoptera acutorostrata*] and 51% Southern Hemisphere [*B. bonaerensis*], all studies combined). In addition, most of the surveys of Japanese markets have revealed evidence of dolphins and porpoises (13% of the total set of 817 samples identified as cetaceans), beaked whales (8%), fin whales (*Balaenoptera physalus*, 5%) and other baleen whales (2%). The Japan surveys included collections organized by Earthtrust, Whale and Dolphin Conservation Society, TRAFFIC Japan, the Fisheries Agency (Government of Japan), IFAW, and Greenpeace Germany. An analysis of the combined samples found significant trends in grouped species composition over time, but no significant differences were found in species composition between collection agencies. The results of the surveys from each collection agency are thus broadly similar although the market composition is changing over time.

[1] See Appendix 7 for a glossary of technical terms.

Genetic testing can help determine whether a specific whale product is legal or not. However, a final determination can be confounded by the inability to verify that the product came from a legitimate source exempted from the IWC's current moratorium on commercial whaling. Legal sources of whale products in Asian markets include: (1) the continuing Japanese catches of minke whales in the Antarctic and western North Pacific under scientific research permits issued by the Government of Japan, (2) the continuing directed catches of Baird's beaked whales (*Berardius bairdii*) and other toothed whales (not including sperm whales [*Physeter macrocephalus*]) in Japanese coastal waters, (3) the cetaceans that are taken in fishing gear as a bycatch (gray [*Eschrichtius robustus*], humpback, Bryde's and sperm whales have occasionally been reported in the Japanese bycatch statistics), and (4) the frozen stockpiles of baleen whale meat and blubber obtained before the IWC moratorium came into effect or obtained from Iceland's scientific research catch between 1986 and 1989. Also, the Bryde's, minke and sperm whales taken through 1987 by Japan's coastal whaling operations under objection to the IWC moratorium could have contributed to the early market samples.

In all market surveys thus far, species identification has been accomplished by estimating the genetic similarity of market samples to sequences obtained from samples of known species or stock origin, i.e., the reference, or "type," samples. The strength of conclusions about identification (i.e., the degree of confidence in the species identification) depends on the degree of genetic differentiation within and between species and on the adequacy of the library of type sequences. It was agreed by the workshop participants that identifications reported thus far in studies of Asian market samples have been accurate, although in some cases, individual samples were not identified to the species level (e.g., dolphins, porpoises, and beaked whales). In some recognized cases, identification of species was complicated by unresolved issues in systematics (e.g., how many species of "Bryde's" whales actually exist), low inter-specific vs. high intra-specific genetic variation for certain taxa (e.g., certain dolphins), and hybridization (particularly the problem of fin/blue whale hybrids that were included in the stockpiled meat from Iceland sold to Japan).

Any comprehensive management scheme for future commercial whaling will likely include provisions for monitoring to detect illegal catches. Genetic tagging of legally taken whales, coupled with comprehensive market surveys, can contribute to such monitoring.

The following recommendations were made by the workshop. Their order of presentation follows the agenda and has no relation to importance or priority.

Recommendation 1: Assigning Identities to Problem Taxa. The workshop recommended that assignments of market specimens to species be made with caution in cases with both large intra-specific diversity and small inter-specific differences, preferably by estimating probabilities of membership in candidate species rather than making an unqualified assignment to the most closely related individual species.

Recommendation 2: Specifications for Public Databases. In order to ensure that public sequence databases (DNA DataBank of Japan [DDBJ], GenBank of the National Center for Biotechnology Information, or European Molecular Biology Laboratory [EMBL]) are accurate and fully documented, the workshop recommended that past submissions be checked and validated; novel sequences be deposited in a timely manner; and new submissions be deposited with the following auxiliary data: sampling location and time, how the sample was procured (e.g., biopsy, bycatch, stranding, etc.), age/maturity status/total length, sex, reference number of the donating tissue archive, basis of species identification (i.e., morphological or genetic evidence).

Recommendation 3: Improvements in Identifying Market Samples, including to the Population Level. Recognizing that adequate reference material is in many cases lacking, the workshop recommended that a hierarchical approach be taken to the identification of market samples. This hierarchy would be organized as follows: identification to family, genus, species, ocean basin, highly distinct population segments, and, finally, stocks. As one proceeds down this hierarchy, the number of reference samples required increases rapidly. To meet this requirement, the workshop recommended that global sampling of reference material be substantially increased and a mechanism be put in place to allow efficient sharing of genetic data.

Also, recognizing the importance of identifying market samples not only to the species level but also to the level of geographical population, the workshop recommended that more effort be expended to gather both tissue samples and sequence data from geographical populations of some baleen whales and almost all toothed whales.

Recommendation 4: Reducing Taxonomic Uncertainty. Noting that the species-level taxonomy of several cetacean groups, particularly the Bryde's/sei whale complex and the minke whales, is unsettled, and recognizing that molecular analyses cannot consistently provide reliable species identifications of tissue samples from such groups in the absence of well-resolved taxonomy, the workshop recommended that a high priority be given to resolving these taxonomic uncertainties.

Recommendation 5: Better Sampling of J Stock Minke Whales. In light of the decline and small current size of the J stock of minke whales (Sea of Japan-Yellow Sea-East China Sea stock), and the lack of thorough knowledge of total annual removals, the workshop wished to express support for the concerns raised at the 51st meeting of the IWC Scientific Committee (1999) concerning removals of J-stock minke whales. The workshop specifically recommended the initiation of biopsy sampling of J stock for DNA analyses and the continued collection of samples from bycatch, strandings, and market surveys.

Recommendation 6: Improving Statistical Techniques. With regard to the need for better documentation of confidence in forensic results, the workshop recommended that, in addition

to increasing samples in and improving access to global reference databases, old statistical techniques be tuned and new ones developed to address forensic questions specifically.

Recommendation 7: Mandatory Sampling of Whales that Enter Commerce. The workshop recommended that tissue samples from all whales destined for the marketplace, including those taken in whaling operations, as well as incidental takes and stranded animals, should be available for verification of specimen origin and for other management-related research. The same should apply to animals that were sampled when caught and whose products may still be in stockpiles.

Recommendation 8: Monitoring Markets that Sell Whale Products. The workshop recommended that monitoring of whale meat in the marketplace be continued and expanded to address a variety of issues, questions, and problems, including: providing information that could be relevant to implementation of the IWC's revised management scheme (RMS), for example on the occurrence in the marketplace of J and O stock minke whales from the western North Pacific; monitoring the appearance in the marketplace of rare or protected species; and identifying new conservation problems that might arise. The workshop emphasized that market sampling designs will vary, depending on the primary issue, question, or problem under investigation.

Recommendation 9: Specifications for Reporting on Species Identification. The workshop recommended that procedures for determining species identities be reported explicitly, including: the genetic marker on which the species identification was based; primer sequences; decision criteria; and sources of reference sequences used for comparison (e.g., a GenBank accession number or, if unpublished, the researcher's name and contact details).

Recommendation 10: Long-term Tissue Sample Storage. Since tissue samples collected from cetaceans in most cases are extremely valuable or even irreplaceable and losses costly or catastrophic, the workshop recommended that such samples held for the purposes of research and management should be stored in duplicate and separate locations for the sake of long-term preservation.

Recommendation 11: Comprehensive Catalogue of DNA Tissue Samples. The workshop recommended that a comprehensive global catalogue of existing cetacean tissues held for genetic analysis be produced and updated on a regular basis.

Recommendation 12: Submission of Market Survey Results to the IWC Scientific Committee. The workshop concluded that within the context of the IWC, the Scientific Committee is the most appropriate forum for considering and evaluating the results of marketplace surveys of cetacean products. Therefore, the workshop recommended that such results be submitted directly to the Scientific Committee.

Recommendation 13: Controlled Access to Genetic Databases. The workshop recommended that a verifiable mechanism be developed to allow controlled access to genetic databases compiled for research and management purposes from scientific and commercial catches, biopsy programs, stranding networks and museum collections.

Recommendation 14: Design Specification of Controlled Access Databases. As a first step in the implementation of a controlled access database, the workshop recommended that a group of interested and qualified scientists be convened to establish the design specifications. The design specifications should cover aspects such as database structure and format, user interface, verification and security safeguards. Safeguards must prevent unauthorized access to the database itself and control the information that is sent in response to queries. The owners of private individual or proprietary national sequence databases must be confident that their interests will be protected.

[This page intentionally left blank]

1. INTRODUCTION

1.1. Opening of the Workshop

The workshop was held at the Southwest Fisheries Science Center (SWFSC) in La Jolla, California, 14-16 June 1999. It was convened by Baker, Dizon and Lento with the assistance of Steering Committee members Brownell, Funahashi, Papastavrou, and Reeves (Appendix 1). Meeting logistics were organized by Lento and Dizon. The workshop was co-sponsored by the International Fund for Animal Welfare (IFAW), the SWFSC, and the World Wildlife Fund (WWF).

Dizon, as the local convener, welcomed participants to the SWFSC, where he heads a program of research on the molecular ecology of cetaceans. It is important to acknowledge the hard work of the Steering Committee in planning and conducting the workshop, and the other participants (Appendix 1) in preparing working papers and engaging in fruitful, open, and sometimes intense discussions. Thanks also to Betsy Douglas (IFAW), Jessica Lipsky (SWFSC), and Aviva Rosenberg (SWFSC) for their essential roles in getting the people to the meeting, feeding and entertaining them while they were there, and dealing with the piles of paperwork. Jay Barlow (SWFSC) and Barbara Taylor opened their house for a traditional southern California "winding down" party after the meetings were over. Finally, we wish to thank Nic Davies for his diligent copy editing of this report. Any mistakes remaining are ours not his.

1.2. Appointment of Chairman and Rapporteurs

Reeves chaired the workshop, assisted by the following individuals who acted as rapporteurs: Baker, Cipriano, Cooke, Dizon, Lavigne, Lento, Palsbøll, Papastavrou, and Taylor.

1.3. Chairman's Remarks

Since the moratorium on commercial whaling came into effect in 1986, a series of studies have been conducted to identify the species composition of whale products for sale in East Asia (Baker and Palumbi, 1994; Baker *et al*., 1996a, b; Lento *et al*., 1997; Cipriano and Palumbi, 1997; Japan Fisheries Agency, 1997; Lento *et al*., 1998a, 1998b; Phipps *et al.*, 1998; Baker *et al*., 1999; Congdon *et al*., 1999; Cipriano and Palumbi, 1999b; Grohmann *et al*., in press). Different investigators have used a variety of techniques to sample markets and to assign samples to species and geographical origin, and *occasionally* to individual animals (Lento *et al.*, 1998a; Cipriano and Palumbi, 1999a).

Species identifications have been based primarily on comparisons of mitochondrial (mtDNA) control region sequences between unknown (or "test") samples and reference (or "type") samples. The majority of molecular species identifications have been based on a phylogenetic approach, using the inferred evolutionary pattern ("tree") of test and reference sequences. Closely related sequences form neighboring clusters, and the position of the test sequences within the branches relative to the reference sequences establishes the species identity. A second approach presented at the workshop relies upon a "near exact match" between a test and a reference sequence to establish the species identity. By either approach, the establishment of species identity is usually reliable providing that an adequate

library of reference sequences is available. However, for some species or species complexes (e.g., the delphinids: *Stenella*, *Tursiops*, and *Delphinus*) the pattern of genetic variation in the control region makes identification to the species level difficult, and sequence data from a different genetic locus, e.g., the mitochondrial cytochrome *b* gene, may have to be employed.

In most cases, the subspecific, stock, or population identity of a market sample is unlikely to be established unequivocally, regardless of the marker employed (nuclear or mitochondrial). With genetic information, one can answer questions regarding the overall statistical differences between, say, two sampling strata (i.e., whether a species is subdivided into stocks). However, even if it is well known that a given species comprises two distinct stocks, shared haplotypes, alleles, or both, usually prevent assignment of single individuals to stock with high statistical confidence. However, nuclear genotyping can determine with a high degree of confidence whether two samples came from the same individual. Questions that can be readily answered include, "How many individual whales are represented in this market sample (Lento *et al.,* 1998a)?" Also, "Is this tissue sample, collected in an Asian market, from a whale that was killed and genetically sampled in the Atlantic Ocean (cf., Cipriano and Palumbi, 1999a)?"

The International Whaling Commission (IWC) is currently developing a Revised Management Scheme (RMS) to regulate future whaling activities. In 1999, the IWC adopted a resolution on DNA testing that calls for the development of scientific advice on matters related to molecular genetic identification of whale products in markets (Appendix 2). This resolution followed an earlier (1997) one encouraging contracting governments to provide information on frozen stockpiles of whale meat and other products and to collect and inventory samples of skin and meat for DNA identification from all whales that enter into commerce (Appendix 2). The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) currently prohibits international trade in all baleen whales and several toothed whales (e.g., sperm whales). However, in 1994 and 1997 proposals were submitted by Parties to downlist various populations of whales and thus allow the resumption of international trade in their products. These proposals were unsuccessful, but similar proposals will certainly be considered by CITES in the future.

This workshop was convened to :

- Review and summarize the results of forensic studies of cetacean products in Asian markets since the international moratorium on commercial hunting of certain species took effect in 1986;
- Review the current scientific and technical methods for molecular identification of cetaceans, including consideration of progress made with other taxonomic groups;
- Identify and evaluate new analytical approaches that could be of use in the molecular identification of cetaceans;
- Review and evaluate methods used to sample product markets;
- Review the status of reference collections and databases and consider ongoing needs to procure and gain access to genetic information.

The draft report was reviewed and agreed on by participants before the workshop adjourned. It was edited in the weeks following the workshop, and a final draft was then circulated to the participants for review and revision prior to printing.

The Steering Committee selected and invited participants to ensure that relevant types of expertise would be represented at the workshop (Appendix 1). Invitations went to scientists from Canada, Germany, Hong Kong, Iceland, Japan, Mexico, New Zealand, Norway, Philippines, Republic of Korea (Republic of Korea (South Korea)), United Kingdom, and United States (Appendix 1). All participants were encouraged to provide working papers, and some were asked to address specific topics in the agenda. A list of working papers is given in Appendix 3; these papers may be available from the authors at their discretion.

1.4. Adoption of Agenda

The agenda, a draft of which had been circulated in advance by the Steering Committee, was adopted (Appendix 3).

2. REVIEW OF MOLECULAR IDENTIFICATION OF CETACEANS: SELECTED CASE STUDIES

Three ongoing case studies were presented for a broad overview of the issues being addressed and the methods employed.

2.1. Baleen Whales in Commercial Markets

Baker reviewed studies of samples collected in commercial markets of Japan and South Korea. To date, at least 11 reports have been published or formally presented (e.g., as submissions to the IWC Scientific Committee) describing surveys of commercial markets for cetacean products in Japan and South Korea (Baker and Palumbi, 1994; Baker *et al*., 1996a, b; Lento *et al*., 1997; Cipriano and Palumbi, 1997; Japan Fisheries Agency, 1997; Lento *et al*., 1998a; Phipps *et al*., 1998; Baker *et al*., 1999; Cipriano and Palumbi, 1999b; Grohmann *et al*., in press). Collectively, these surveys have determined the identities of nearly a thousand cetacean products (Table 1).

Molecular genetic surveys of whale markets began in 1993 at the initiative of Earthtrust, an NGO based in Hawaii (Baker and Palumbi, 1994). The initial goal was to identify the species origin of cetacean products purchased on the commercial markets of Japan. The 1993 study adapted existing molecular technology for use in the field. This allowed species identification of 18 of the 41 product samples collected that year. The diversity of species represented in this initial sample was surprising and provided the impetus to expand sample collection and to improve field extraction and Polymerase Chain Reaction (PCR; Mullis and Faloona, 1987) amplification methods. A subsequent market survey, co-sponsored by Earthtrust, IFAW, Earth Island Institute, and others was carried out in South Korea in 1994. Most of the samples purchased from commercial markets in Japan and Korea were sold as "kujira," the generic Japanese term for whale or "gorae," the generic Korean term. In both countries, most of the samples were from baleen whales.

A number of regulatory, technical and biological problems were encountered during the market surveys. Compliance with CITES

regulations required the development of portable equipment so that analyses using the PCR could be conducted in the field (Bowen and Avise, 1994; Jones, 1994). Verification of the experimental methodology was an important consideration. Methodological considerations have included the choice of appropriate phylogenetic methods, the choice of appropriate molecular markers, and the possibility that cross-contaminating PCR artifacts, and amplification of pseudogenes could lead to erroneous or misleading results. Analytical considerations have included the uncertain or incomplete taxonomy of baleen whales, the adequacy of reference databases, the adequacy of market sampling, the need to analyze mixed-species products, identification of hybrids, and the need to identify geographic or stock origins of products. More recently, the need has been identified for both field-based and laboratory-based rapid species typing to allow a high sample throughput.

2.2. Toothed Whales in Bycatch

Chivers described the use of the SWFSC's reference database to confirm field identifications of toothed whales taken incidentally in California gillnet fisheries. Cetaceans killed in these fisheries that are known to be difficult to identify to the species level include common dolphins (*Delphinus* spp.), dwarf (*Kogia sima*) and pygmy (*K. breviceps*) sperm whales, and beaked whales. She emphasized that for these groups, wherever possible, morphological features were primarily and preferentially used for species identification. Species assignments, at least among some of the delphinids, based on genetic data alone is difficult or problematic due to the overlap of intra-specific and inter-specific variability in the control region. A large number of reference sequences are required for each taxon. For example, species-specific PCR primer methods were originally used to distinguish the two species of common dolphins (short-beaked, *Delphinus delphis* and long-beaked, *D. capensis*). The approach was based on the results of Rosel *et al.* (1994), which showed two fixed nucleotide substitutions between the long-beaked and short-beaked common dolphins. However, Chivers noted that when the sample size was increased to more than 100 individual sequences, those formerly fixed differences disappeared. The cytochrome *b* gene is now used because, thus far, it unambiguously distinguishes these two species.

The species identifications based on morphology were made by either Susan Chivers or John Heyning (Los Angeles County Museum) using either the carcass, the head or good-quality photographs. To date, there has been 100% agreement between these identifications and those from DNA data. Mis-identifications by fishery observers occur due to inexperience or in situations involving juveniles or females (in the case of beaked whales) or badly decayed remains. In those cases, genetic identification has proven very useful.

2.3. Strandings of Beaked Whales

Dalebout described the molecular genetic identification of stranded beaked whales (Ziphiidae) in New Zealand. Species identification of beaked whales based on morphology is difficult. The main diagnostic feature is the number, shape, and placement in the jaw of the teeth, which erupt only in adult males. Females and juveniles may be nearly impossible to identify without dissection to observe cranial features, and this is obviously impossible with live strandings. Several beaked whale species are still only known from strandings or fragmentary skeletons.

New Zealand has an exceptionally high frequency and diversity of beaked whale strandings. Control region and cytochrome *b* sequences have been used to develop a database for identification of beaked whale species (Dalebout *et al*., 1998). This database is also used to study the phylogeny of that family, and many entries are supported by museum records and morphological data. The database presently has representation from 18 of 21 recognized species, including the published sequences from Henshaw *et al*. (1997).

In New Zealand, the rate of mistaken field identifications by field collectors has decreased from 20% (n=40) for the first five years of the database to 7.5% (n=97) for the nine different species collected from strandings since. This trend was attributed mainly to increased vigilance by collectors as they have come to appreciate how difficult it is to identify beaked whales.

Uncertain taxonomy may be a concern for accurate identifications of the family because several new species have been described in the last 10 years. The potential was evaluated for overlap in the ranges of intra-specific and inter-specific differences among the species of the family. Only the intra-specific average within the southern bottlenose whales (*Hyperoodon ampullatus*) approached that of inter-specific averages within the family. However, reviews of the genetic information and stranding records have revealed evidence for a new species that is morphologically similar to Hector's beaked whale (*Mesoplodon hectori*; Dalebout *et al.*, 1998; Henshaw *et al.*, 1997).

3. LESSONS FROM MOLECULAR MONITORING IN OTHER TAXA

Molecular techniques have been used to identify other wildlife products in the marketplace. In order to benefit from the experiences gained with monitoring the trade in other taxa, contributions were requested from experts on turtles, sturgeons, and seals.

3.1. Turtles

Bowen summarized the molecular monitoring work that has been conducted on turtles and turtle products. Results of such studies have been used to influence both domestic and international policy. For many marine turtles, nesting beaches are genetically distinct and can be treated as separate management units. These strong genetic differences have allowed evaluation of anthropogenic mortality on feeding grounds, which are usually characterized by representation from multiple breeding beaches. Mixed-stock analyses, which were developed to apportion high-seas fishery harvests of salmon to different rivers (Pella and Milner, 1987), are used to estimate kill levels for different nesting beaches.

Forensic approaches have been used to identify freshwater turtles sold in markets of some states within the U.S., and the results have influenced domestic policy (Roman and Bowen, in press). Although no illegal products were found in one market survey, meat was frequently mislabeled. Results also showed that the sale of alligator snapping turtles (*Macroclemys temminckii*) had declined and that these large turtles were being replaced by the smaller common snapping turtle (*Chelydra serpentina*). Bowen noted that turtles followed the pattern typically seen in over-exploited taxa:

as large commercially valuable species become depleted, they are replaced by smaller, but similar, species. The final step in this progression is gross mislabeling. In his example, 22% of products identified as turtle were in fact alligator (*Alligator mississippiensis*; Roman and Bowen, in press).

Mislabeling is a common practice in the caviar markets. In 1 study, 5 of 25 products sampled were mislabeled, and in 2 of those, the products proved to be from species listed as threatened or endangered (DeSalle and Birstein, 1998). Thus, a legal trade can conceal illegal products.

Dutton pointed out that turtle nesting beaches in the eastern Pacific are not as clearly genetically distinct as those in the western Atlantic that Bowen used as examples. Two beaches may be demographically separate, at least judging by lack of movement by tagged animals between nesting beaches, yet turtles from the two areas are genetically indistinguishable. Thus, a mixed stock genetic analysis will not be effective on the high seas for these populations. This problem seems to apply particularly to leatherback turtles (*Dermochelys coriacea*), which have low genetic diversity in mtDNA, necessitating the development of more sensitive markers.

3.2. Sturgeons

Although a geneticist from the U.S. Fish and Wildlife Service Forensic Laboratory was expected to discuss sturgeons (agenda item 3.2), he was unable to attend at the last minute. It was recognized that considerable progress had been made in monitoring the trade in caviar using molecular identifications (DeSalle and Birstein, 1996).

3.3. Pinnipeds

Lavigne reported on progress to monitor pinniped penises in the marketplace (see Malik *et al.,* 1997). Although penises are marketed as aphrodisiacs in several processed forms, including pills, powders, and even wines (alcohol extracts), DNA could only be extracted from unprocessed penises. Ethnic Asian markets were surveyed in Canada, the U.S., and the U.K. Agents of Asian descent were employed to reduce suspicion on the part of shopkeepers. Sampling was opportunistic because finding the samples was unpredictable. Also, because the penises were often expensive, if there was a choice between two similar-looking penises and two different-looking penises, the latter pair was purchased. This research was exploratory only, but it could facilitate the development of a systematic sampling design in the future.

Because of the pinniped harvest in Canada, seal penises were readily available there (8 of 12 stores surveyed). Of 15 samples, 9 were from harp seals (*Pagophilus groenlandicus*) or hooded seals (*Cystophora cristata*) and the rest from mammals other than pinnipeds. In the U.S., where it is illegal to sell marine mammal products, seal penises were on sale in 35 of 72 shops visited. Of 16 purported seal penises, only 1 was genuine; it was from a fur seal, *Arctocephalus pusillus*, either from Australia (the Australian fur seal, *A. p. doriferus*) or from southern Africa (the Cape fur seal, *A. p. pusillus*); the rest were from other mammals.

Limited surveys have been conducted opportunistically in China and Thailand. In China, 12 supposed seal penises were purchased, and, indeed, the sample comprised 11 harp or hooded seals and 1 Australian or Cape fur seal. In Thailand, three putative seal

penises were purchased; one was identified as harp seal and the remaining two as other mammals. No penises of northern fur seals (*Callorhinus ursinus*) were identified despite the fact that they are known to be marketed in China. Advertisements were found for this species and for the highly endangered Hawaiian monk seal (*Monachus schauinslandi*). The dealer advertising monk seal products claimed to be out of stock.

Some of the specimens fraudulently labeled as seal proved to be from gray wolves (*Canis lupus*) and African wild dogs (*Lycaon pictus*), both of conservation concern, especially the latter.

4. METHODS FOR MOLECULAR IDENTIFICATION OF SPECIES

The process of making identifications to the species level is well established, and confidence in the identifications is generally adequate. For the most part, the control region of the hypervariable 5' end of the mtDNA genome is employed although cytochrome *b* may be a better choice for beaked whales. Confidence in species identifications depends on the adequacy of the library of type sequences. Although the total number of type specimens held in laboratories and public sequence databases around the world is impressively large, the taxonomic coverage is very patchy in terms of adequately representing the range of intra-specific geographic diversity for most species. In addition, in many cases there is incomplete documentation of the sample itself. Because of the high value of reference libraries of type sequences, steps must be taken to broaden their coverage in terms of the number of different species included and the number of sequences for a given species, strengthen their documentation in terms of how field identifications were made prior to sampling, and improve their general availability to investigators from other laboratories (see 7.1 and 7.2, below).

4.1. Phylogenetic Methods, Confidence, and Consistency of Identifications

Species identifications of whale products in commercial markets in Japan and South Korea have relied primarily on phylogenetic reconstruction of DNA sequences. This approach is based on the comparison of test DNA sequences (usually the variable segment of the control region) to type sequences from a library of sequences from well-documented specimens that span the taxa of interest (Fig. 1). The steps involved in phylogenetic identification are (1) amplification via PCR and direct sequencing of the target regions, (2) alignment of test and reference sequences, (3) comparison of sequences by phylogenetic reconstruction and (4) measurement of reliability or consistency of groupings among reference and test sequences, usually by bootstrap re-sampling procedures. The reconstruction is usually represented as a "tree," with closely related sequences forming neighboring branches. Identification to species is based on the grouping of a test sequence with type sequences from one species, to the exclusion of other type sequences from other species, and some level of bootstrap support for this grouping. A reconstruction can be based on genetic distance (i.e., the number of nucleotide differences between two sequences) such as the

neighbor-joining method or on character-based approaches such as parsimony or maximum likelihood. The latter two approaches are more computationally intensive but may perform better across a broad range of conditions encountered in sequence evolution. For identification of baleen whale products, the neighbor-joining method has usually proved adequate but is often verified with parsimony

The strategy for the phylogenetic identification of cetacean products involves a hierarchical comparison to establish, first, the suborder and family using a small number of reference sequences from a large number of species. Next, the test sequence is compared to a large number of reference sequences from one or a few of the likely species, as indicated by the initial analysis. One or more outgroups (i.e., more distantly related species) are used to protect against a mis-classification error. As a conservative approach to identification, Baker *et al.* (1996a, b) recommended that species identification should be considered "confirmed" only when a test sequence has nested within the range of type sequences of a given taxon (i.e., species). If the test sequence has not nested but is intermediate in position between two clusters of taxa, it could represent an outlier (in terms of genetic distance) of either of those two taxa. It could also belong to a related intermediate taxon not included in the type sample. Such a pattern was recognized in a product purchased on the Korean market in 1994 and inferred to have been a pygmy Bryde's whale (Baker *et al.,* 1996a, b).

Bootstrap re-sampling is a statistical method that can be used to assess the strength of support for the grouping of a test sequence with type sequences of the most closely related species relative to other clusters in the tree. Statistical support for an identification of a test sequence can be estimated by bootstrap re-sampling of the sequence data using either (or both) parsimony and distance methods. Parsimony analysis, although slower than distance methods, includes consideration of particular phylogenetically informative sites and is therefore preferred for assessment of bootstrap support for grouping hypotheses. In some cases (e.g., *Stenella*, *Tursiops*, and *Delphinus* species), control region sequences show high variability within species and low divergence between species, few or no diagnostic (fixed) differences between species, and, as a result, bootstrap values are typically low. It is then difficult to place much confidence in the assignment of these sequences to particular species. In other words, the phylogenetic relationships of the species within such closely related groups are difficult to reconstruct. In such cases, the phylogenetic approach is not useful for precise species identification of test samples, and investigators may simply have to look for a match between the test sequence and one of the type sequences. For this to be effective, a very large library of type sequences of the difficult groups of taxa is required to increase the probability of finding a match.

The problem of high intra-specific variability and low inter-specific divergence has not been encountered in identifications of baleen or beaked whales. When problems have been encountered with these taxa, it has been due to incomplete taxonomy or the absence of reference sequences (e.g., the pygmy Bryde's whale, see above).

For the dolphins, species identification at the SWFSC is based on searching for a match between the sequence of the unknown and one of the sequences in the reference library. As described by Dizon, the search does not require an aligned test file of all test and reference

individuals that is required with phylogenetic reconstruction. Rather, it involves a simple pairwise alignment of a given test sequence with a single reference sequence in the library. The unknown sequence is aligned individually with each one of the reference sequences, the goodness of the match is reported for each alignment. An unknown is considered identified if an identical or almost identical match is found. The pairwise alignment and scoring procedure is implemented by a computer program developed at the SWFSC, "MacMatch," and is similar in practice to the process of searching international sequence databases (DDBJ, EMBL and GenBank).

One advantage of this approach is that it obviates the need for constructing large, globally aligned datasets that include the unknowns themselves, a process that is laborious. With the phylogenetic approach, the dataset sometimes has to be re-aligned when a new test sequence appears that contains previously unobserved insertions. A second advantage is that test libraries can be easily shared between laboratories, because re-aligning of datasets by each laboratory to include their new test sequences will not be required. Once the libraries are constructed and verified they do not have to be changed; testing can then be standardized across laboratories. Finally, the reliability of the process can be evaluated by a jack-knife re-sampling procedure that may be simpler to implement than the bootstrap approach.

Dizon illustrated how "near" a "near exact match" has to be with three of the problematic taxa within the delphinids: *Stenella*, *Tursiops*, and *Delphinus*. For some species pairs in this complex, fewer than 4 base pair differences are required for a 400 base pair control region sequence. Among these taxa, the control region sequence of a given member of one species may be more genetically similar to a given member of a different species than it is to some conspecifics. To help reduce mis-classifications due to this large and at times overlapping level of genetic diversity, a large library of reference samples is required. Details about the use of both the phylogenetic approach and the searching-for-matches approach for several delphinid products of unknown origin are presented in Appendix 4.

In considering a statistical test of species identification by simple matching, several considerations were raised. One was the question of independence of site variation in the matching approach. Would this be a violation if matching were used in a statistical approach? Another potential limitation of matching is its dependence on a large reference dataset that might not be available for rare or inaccessible species. However, it was acknowledged that in the case of extensive overlap in inter-specific and intra-specific variability, large datasets would be required for an attempted identification.

The workshop then considered some biological situations of experimental artifacts that could complicate molecular monitoring. Kim presented the observation that heteroplasmy as well as nuclear translocations of mtDNA (Numt's) are commonly observed in vertebrates (Lopez *et al.*, 1994). These factors have the potential to introduce error in subsequent data analyses at all phylogenetic levels if ignored. Numt's have mutation rates comparable to the mutation rates of nuclear operons. Thus a relatively low (approx. 10 times lower) degree of diversity is typically observed at such loci. One could consider these loci to be molecular "fossils," in a sense. While unrecognized Numt's are likely to yield highly

erroneous results, such sequences, when identified, might also provide valuable insights with respect, for example, to molecular evolution.

The group noted that heteroplasmy was unlikely to cause mis-assignments at the species level, as heteroplasmic changes typically involve only a very few base pairs. Similarly, the group noted that Numt's were a concern but did not appear to be a pressing issue. Although some species display unexpectedly low levels of diversity, none of the signs associated with a nuclear translocation had been noted. Typical indications of the possible presence of a Numt in the coding region include insertions/deletions, an atypical transversion/transition ratio, an atypical synonymous/non-synonymous mutation ratio, and inappropriately positioned stop codons. The presence of Numt's in both coding and non-coding regions could be signaled by indications of heterozygosity at variable sites, giving rise to double bands in PCR amplifications or double peaks on SSCP genotypes or in sequencing electrophoretograms. As double bands/peaks in mtDNA sequences are possible indications of heteroplasmy or the inadvertent amplification of nuclear translocations, the workshop noted that attention should be directed toward identifying double bands/peaks. Lento and Dalebout noted that such bands occur in control region amplifications of Dall's porpoise (*Phocoenoides dalli*).

Daníelsdóttir pointed out that species identifications based solely upon maternally inherited mtDNA sequences will assign hybrid individuals to the maternal species. It was suggested that the problem for putative blue/fin whale hybrids could be addressed in the field. Species-specific primers that identify market samples to either fin or blue whales can be adapted for use in the field (see below). Subsequent amplification of, e.g., α-lactalbumin (Bérubé and Aguilar, 1998) introns or actin I introns (Palumbi and Baker, 1994; Cipriano and Palumbi, 1999a), followed by restriction endonuclease digestion, will allow identification of blue/fin whale hybrids.

4.2. Comparative Power of Molecular Markers and Adequacy of Type Databases

Dalebout discussed the consistency and sensitivity of the control region and cytochrome *b* for species identification of beaked whales. The control region is usually the preferred marker for species identification and investigation of population structure in cetaceans because of its rapid rate of evolution (Dizon *et al.*, 1997). Cytochrome *b* has also been used in some studies, but because it is a protein-coding locus, its evolution is constrained by the need to produce a functional molecule. Cytochrome *b* is commonly much less variable than the control region, with most polymorphic sites occurring at the third codon position (i.e., synonymous substitution). However, whereas the majority of variable sites in the cetacean control region tend to be clustered in the first 200 base pairs of the 5' end, informative sites may be more widely and evenly distributed in cytochrome *b*.

While recognizing that the control region is well suited to answer questions about species identity for most cetaceans, other loci may offer advantages for certain groups. Dalebout showed that both loci are suitable for phylogenetic identification of beaked whales, but cytochrome *b* may be preferable due to its high inter-specific variation and wide distribution of variable sites in comparison to the control region. Cytochrome *b* may also be useful for the problematic delphinids in which the more

rapidly evolving control region can mask species distinctiveness due to high intra-specific and relatively low inter-specific variation at this locus.

Recommendation 1: Assigning Identities to Problem Taxa. The workshop recommended that assignments of market specimens to species be made with caution in cases with both large intra-specific diversity and small inter-specific differences, preferably by estimating probabilities of membership in candidate species rather than making an unqualified assignment to the most closely related individual species.

The group discussed the importance of detail and quality control in constructing and adding to sequence databases. Recognizing that embedded errors in species identification can self-propagate and compromise future species assignments, it was agreed that, at a minimum, type sequences should be distinguished from test sequences (sequences identified to species based on comparison with type sequences). As all recognized cetacean species were originally described based on morphological characters, the best type sequence is one from a sample vouchered by a museum specimen identified by an expert in cetacean taxonomy or from a photograph showing diagnostic characters. A sequence from a market sample or biopsy from an animal must be considered to be potentially less reliable. Thus, inclusion of information on the provenance of a type sample and any voucher material will contribute to the confidence in a species assignment based on it.

Lento presented a summary of the reference sequence database held at the School of Biological Sciences, Auckland University. The database contains approximately 850 sequences. She briefly outlined the procedure employed to determine the species identity and, when possible, the geographic origin of test samples. In total, three phylogenetic estimations based upon control region sequences are performed on an increasingly finer phylogenetic scale. The primary analysis includes the test sample plus 50 reference samples, representing all cetacean families. The outcome of this primary analysis determines the taxa to be included in the secondary identification. For instance, if the test sample clusters within the toothed whales, the second analysis would include the test sample plus some 64 toothed whale sequences. Once the species is determined, the final (tertiary) analysis aims at determining geographical origin, in cases where representative samples from different populations are available and those populations that are genetically distinguishable.

Cipriano summarized the reference database at the Center for Conservation and Evolutionary Genetics, Harvard University. Species assignment of test samples is based upon control region sequences as above. The database includes 314 reference sequences (192 from 15 baleen whales and 122 from 40 toothed whales). A supplemental cytochrome *b* dataset contains 85 reference sequences (12 from 12 baleen whales and 73 from 32 toothed whales).

The reference database at the Japan Fisheries Agency (JFA) was summarized from an IWC document (IWC/49/INF3). It contains 34 control region sequences (21 from baleen whales and 13 from toothed whales).

Dizon summarized the SWFSC's reference sequence database, which consists of 2,316 sequences (2,094 from toothed whales and 222 from baleen whales) and 299 cytochrome *b* sequences (9 from baleen whales and 290 from toothed whales). In total, the database contains

sequences from 10 baleen whale species and 60 toothed whale species. Former test samples are included in the database, although the total number of test samples is fewer than 100. Details on the origin of each sample (e.g., marketplace, necropsy, biopsy, stranding, incidental take, and other pertinent details) are maintained in a linked database. Dizon made the point that since the SWFSC makes its identification based on matches, it is relatively easy to provide information about the provenance of the sequence or sequences that matched the unknown sequence without qualifying the sequence as "reference." He felt that the qualifying process could at times be arbitrary because not every reference sample has come from an animal which has been thoroughly examined by a trained specialist.

Proper representation of intra-specific variation is important, so new reference sequences should be submitted to one of the three international sequence databases in a timely manner. See Appendix 5 for an example of a fully documented GenBank submission. Sequences retrieved from public databases should be checked carefully against the original publication to verify the taxonomic designation and to ascertain how the taxonomic designation was made. In general, relatively sparse auxiliary data are provided with sequences submitted to public databases. Reliable assignments of test samples to species and geographic region hinge critically upon access to a comprehensive and validated set of reference sequences.

Recommendation 2: Specifications for Public Databases. In order to ensure that public sequence databases (DNA DataBank of Japan [DDBJ], GenBank of the National Center for Biotechnology Information, or European Molecular Biology Laboratory [EMBL]) are accurate and fully documented, the workshop recommended that past submissions be checked and validated; novel sequences be deposited in a timely manner; and new submissions be deposited with the following auxiliary data: sampling location and time, how the sample was procured (e.g., biopsy, bycatch, stranding, etc.), age/maturity status/total length, sex, reference number of the donating tissue archive, basis of species identification (i.e., morphological or genetic evidence).

The workshop recognized that high-quality reference sequences are essential for reliable analyses. Inclusion of test samples in a reference database after their identification risks introducing errors in subsequent species/geographic assignments of new test samples. Although inclusion of test samples may be useful for self-referencing purposes, they should not subsequently be used as reference samples for the initial species identification of new test samples.

Morphologically identified specimens are also subject to errors in taxonomic assignment, especially in cases where samples are taken at sea from carcasses, or obtained from biopsy darts. In all cases, the sources of reference materials should be identified in database annotation, or referred to a published paper containing source information, so that provenance of the original identification can be determined.

4.3. Technical Limitations and Advances

Cipriano described a technique being developed at his laboratory that uses rapid identification of known, species-specific polymorphisms to allow high-throughput species identification. The technique (oligonucleotide ligation assay [OLA], Baron *et*

al., 1996) exploits the fact that a *Taq* ligase enzyme will join two oligonucleotide probes annealing at adjacent positions along a test DNA template, e.g., a template such as PCR product from a control region amplification. Species-specific differences in the target annealing regions of the two oligonucleotides will prevent attachment of one or both probes and thus ligation. The ligated oligonucleotide probe pair can be distinguished subsequently from the two smaller, non-ligated probes by electrophoresis. The process can be automated by using fluorescently labeled probes and laser detection electrophoresis. The method also can be multiplexed, allowing screening/identification of several species in a single experiment. Species-specific oligonucleotide probe sets were presented for humpback, fin, and blue whales. Additional probes for minke whales are under development. The accuracy of the procedure has been tested using the same PCR products as are used for sequence-based identifications.

Lento described a multi-step PCR analysis that also enables quick characterization of samples in the field using taxon-specific diagnostic primers (Lento *et al.*, 1997). The method consists of a hierarchical set of PCR amplifications, each with an increased specificity of template. The steps are to discriminate (1) whales from ungulates, (2) minke whales from other cetaceans, (3) northern minke from southern minke (*Balaenoptera bonaerensis*) whales, (4) fin whales from other cetaceans, and (5) blue whales from other cetaceans. The procedure was tested in the field for 76 samples, where subsequently the unknown was confirmed by sequencing. Of these 76 test cases, 63, 7, and 6 cases yielded a correct, false negative, or inconclusive result, respectively. No false positives were recorded.

Both the oligonucleotide ligation and the taxon-specific PCR methods were proposed as ways of rapidly screening samples to aid in establishing priorities for additional analyses. The taxon-specific PCR method has the added advantage of being useful in field settings but requires additional PCR analyses for each contrast. OLA probes can be multiplexed to distinguish multiple species in a single post-PCR reaction. It was noted that both procedures incorporate internal positive controls, which would enable detection of false negatives.

In order to avoid the need to make a choice of markers in the field and to maximize future analysis options, Dizon directed the group's attention to another method known as "whole genomic amplification" (Dietmaier *et al.*, 1999). This method employs PCR amplification with an oligonucleotide primer that is four-fold degenerate at all sites. It aims at generating amplification products that represent most of the genome, which can subsequently be purified and transported across national borders without the need for CITES permits.

5. GENETIC IDENTIFICATION OF GEOGRAPHIC ORIGIN AND "STOCK" IDENTITY

The goal of this section was to examine the present feasibility of identifying market samples to below the species level and to consider the limitations and future prospects of being able to do so. The goal was not to address the very general (e.g., analytical techniques for studying population subdivision or structure) or the very specific (e.g., stock structure in particular taxa). Species-level identification was dealt with in the previous section.

5.1. Stock Definitions under Various Management Schemes

Taylor began by suggesting that identification to stock level may be unnecessary for many forensic cases. Thus, focusing on the "simple" question of "where the sample came from" rather than the more complicated question of "what stock it came from" will preclude considerable controversy. There are exceptions, however, such as the Sea of Japan (J) and Sea of Okhotsk (O) stocks of minke whales, which show nearly fixed differences in the frequencies of mtDNA haplotypes.

Taylor also emphasized that being clear about management goals is a prerequisite for defining stocks and pursuing genetic identification to that level. She contrasted the management objectives related to stock definitions under the U.S. Marine Mammal Protection Act (MMPA) and within the IWC. In the latter, there is little consensus about management goals as they relate to stocks.

The two main objectives of the MMPA are to maintain populations (1) at levels above half of the historical abundance and (2) as functioning elements of their ecosystems. The stock-definition problem has been tied to the second element by the logic that it means ranges must not be reduced or fragmented. Taylor (1997) provided a technique to estimate a level of immigration from adjacent areas (dispersal) required to meet this objective in the face of local anthropogenic mortality. Because relatively high gene flow is expected in this risk-averse definition of stock, dispersal between adjacent populations (i.e., stocks) would be relatively high and genetic diversity low, i.e., characterized by allelic frequency differences rather than fixed ones. Thus, identification of unknown market samples to the stock level would be probabilistic rather than unambiguous.

The IWC has not clearly defined the unit of conservation and continues to struggle with taxon-specific stock decisions. Different lines of evidence have been employed and drawing stock boundaries on maps has usually been contentious. The tendency has been to make large, evenly sized units, divided north to south. The lack of data and the fear of extirpating local populations if errors were made in stock definitions led to the precautionary practice of using "small areas" as the management units within the RMS.

For the purposes of this workshop, the salient point was that within the IWC Scientific Committee, there is no agreement on the population unit to be conserved. Taylor therefore suggested that while stock definitions remain at issue, a hierarchical approach to the identification of market samples would be efficient: identification to species, then to ocean basin, then to highly distinct population segments, and finally to stocks. Confident identification to species is generally reliable given a sufficient type collection. The ease of identification to ocean basin will vary by species. Generally, with low levels of divergence between basins, identification of an unknown to a particular ocean basin becomes problematic.

Assigning individuals to stocks will always be difficult, not only from the technical perspective (low statistical power due to high levels of interchange between adjacent units on an evolutionary scale), but also because the definition of stocks is policy-driven and unresolved in many contexts. Taylor emphasized that many politicians believe that stocks can be defined solely on the basis of

biology (see Taylor and Dizon in press) and are unaware that further policy clarifications are needed.

The goal of the investigation determines the level of identification required. If the issue is whether proscribed species are being taken, the hierarchical approach is unnecessary. If it is important to know where the specimen was taken, the next level of resolution is required. If it is important for management purposes to apportion mortality to stock, one will need to identify the market sample to the stock level.

Recommendation 3: Improvements in Identifying Market Samples, including to the Population Level. Recognizing that adequate reference material is in many cases lacking, the workshop recommended that a hierarchical approach be taken to the identification of market samples. This hierarchy would be organized as follows: identification to family, genus, species, ocean basin, highly distinct population segments, and, finally, stocks. As one proceeds down this hierarchy, the number of reference samples required increases rapidly. To meet this requirement, the workshop recommended that global sampling of reference material be substantially increased and a mechanism be put in place to allow efficient sharing of genetic data.

Also, recognizing the importance of identifying market samples not only to the species level but also to the level of geographical population, the workshop recommended that more effort be expended to gather both tissue samples and sequence data from geographical populations of some baleen whales and almost all toothed whales.

5.2. Phylogenetic and Statistical Methods for Population Analysis

Baker illustrated the utility of a hierarchical strategy for identification of species, oceanic population, and stock for baleen whales using a tree-based phylogenetic approach. As noted previously, most baleen whales can be unambiguously identified to the species level (Baker and Palumbi, 1994; Baker *et al.*, 1996a, b). Bootstrap support of 95-100% is observed for species-level nodes within them. An exception is the Bryde's whale species complex, for which there is uncertainty about the number of species involved.

The next level of analysis requires identification to the ocean basin level. Baker illustrated this with northern minke whales. Virtually all unknown samples from the Korean and Japanese markets were nested within the North Pacific clade when compared to the established North Atlantic clades (Baker *et al.*, 1996a, b). Nodes separating those stocks (subspecies) were distinguished by five fixed nucleotide differences and supported at the 97-98% bootstrap level**,** lending a great deal of confidence in determining the ocean-basin origin of minke samples (Lento *et al.*, 1998b).

For the western North Pacific J and O stocks, the ability to apportion unknown market samples is also high because of large differences between the two stocks in frequencies of control region haplotypes (Goto and Pastene, 1997). Baker argued that, although a single product could not be assigned with certainty, a collection of samples was amenable to statistical testing. This was illustrated with a test of market samples of North Pacific minke whales from Japan compared to the reported scientific catch. Once the differences between a sample and a putative source had been established, a

maximum likelihood approach was used to estimate the contribution of an alternative putative stock (Pella and Milner, 1987). Finally, exclusion of duplicated individuals was confirmed by microsatellite profiling for samples with identical mtDNA haplotypes (Lento *et al.*, 1998a).

It was recognized that stocks of many of the baleen whales and most of the toothed whales are not so well differentiated. Therefore, the ability to apportion unknown samples to stocks or even ocean basins must be evaluated on a species-by-species basis. In the discussion, it emerged that identification of the southern dwarf form of the minke whale remains somewhat uncertain due to the lack of reference material, although this form seems most closely related to the North Atlantic population.

Recommendation 4: Reducing Taxonomic Uncertainty. Noting that the species-level taxonomy of several cetacean groups, particularly the Bryde's/sei whale complex and the minke whales, is unsettled, and recognizing that molecular analyses cannot consistently provide reliable species identifications of tissue samples from such groups in the absence of well-resolved taxonomy, the workshop recommended that a high priority be given to resolving these taxonomic uncertainties.

It was strongly emphasized that obtaining reference samples from the J stock (Sea of Japan-Yellow Sea-East China Sea stock) is necessary in order to estimate accurately the proportion of those animals in the Japanese markets.

Recommendation 5: Better Sampling of J Stock Minke Whales. In light of the decline and small current size of the J stock of minke whales (Sea of Japan-Yellow Sea-East China Sea stock), and the lack of thorough knowledge of total annual removals, the workshop wished to express support for the concerns raised at the 51st meeting of the IWC Scientific Committee (1999) concerning removals of J-stock minke whales. The workshop specifically recommended the initiation of biopsy sampling of J stock for DNA analyses and the continued collection of samples from bycatch, strandings, and market surveys.

O'Corry-Crowe outlined his forensic work with beluga whales (*Delphinapterus leucas*). Native Alaskans are legally entitled to hunt beluga whales throughout the state, and whale products may be bartered among or sold to natives in native villages. Until recently, there were no limits on take. The geographically distinct population of beluga whales in Cook Inlet is small, numbering around 350 animals, and it is believed to have been declining at a rate of 15% per year for the past five years (Hobbs *et al.*, 1999). Thus, there are concerns over the origins of beluga whale muktuk (skin and blubber) sold in a native store in Anchorage, the state capital and largest native village in Alaska.

O'Corry-Crowe described a molecular genetic approach to assign samples of whale muktuk bought in this native store. The goal is to discriminate individual animals to their stock of origin and thus track their post-mortem history. This requires baseline data on multiple genetic loci from large numbers of samples from reference populations or stocks. Questions being specifically addressed include: From which stock or stocks does the muktuk sold in a native store originate? How many whales are involved in this market? With regard to concerns about the reproductive potential of the declining Cook Inlet stock, are both male and

female whales being hunted for market? And which hunters are selling muktuk?

To date, the study has found that mtDNA alone has limited utility for determining population of origin for a test animal. Genetic diversity in mtDNA is low and common haplotypes are shared between well-established stocks. Nevertheless, mtDNA does demonstrate significant genetic subdivision (O'Corry-Crowe *et al.*, 1998). Having only a few common haplotypes limits the ability to distinguish individuals and therefore track or re-identify a particular animal. However, assignment tests based on the eight microsatellite loci so far examined may have sufficient power to determine the stock origin of a sample. At a minimum, it has been possible to discriminate Cook Inlet animals from those taken north of the Aleutians.

5.3. Sample Size and Power

Taylor noted that hypothesis testing involves the comparison of two hypotheses and that statistical power is defined in this context. Because identification of a market sample examines the plausibility that it could be from one of many hypothesized species or stocks, hypothesis testing will rarely provide the appropriate statistical framework. The process of identification could be more accurately characterized as parameter estimation. However, the most important factors that affect power also affect the ability to identify correctly a sample to origin. Those are effect size, i.e., how different "things" are, and sample size. In genetic terms, the effect size is the amount of genetic differentiation between the putative strata. In statistical terms, with high power it would be easy to decide whether a market sample came from one of two statistical populations. When the effect size is very large, precision of the estimate becomes irrelevant because there is no chance of genetic overlap or intra-species and inter-species variation. As effect size decreases, precision becomes critical and affects the ability to distinguish between "hypotheses."

There are a few instances when hypothesis testing could be employed because there are only two hypotheses. For example, the following hypotheses could be framed: H_o: this test specimen comes from J stock, H_a: this test specimen comes from O stock (Okhotsk Sea-West Pacific stock). Presumably, since there is no reason to believe it comes from one stock or the other, the potential errors (falsely rejecting the null or alternative hypotheses) should be set to be equal. Setting the errors may be difficult because the amount of expected difference, the effect size, is likely to be unknown.

Taylor also pointed out that most assignment tests crudely assign a specimen to the stock in which the individual's haplotype is most common. Thus, if a genotype is very common in a rare population and less common in an abundant population, it will assign the test specimen to the rare population even though there are actually more individuals with that genotype in the abundant population. Accordingly, if one knows nothing about harvest, it might be more sensible to assign the individual probabilistically in relation to the number of individuals with that haplotype in different populations. If there is information on harvest, then clearly those data should be used in the probabilistic assessment of the specimen's origin. In either case, the origin of the test specimen is better described by estimates of probability rather than by a simple assignment.

This question of individual assignment is different than the question of apportioning a

given sample to different stocks, e.g., the mixed-fishery analysis problem. In the former, only the distributions of the alleles in the source and the sample populations have to be compared.

For the purposes of monitoring whale markets, this latter approach can be useful. If the null hypothesis that the two compositions are the same is rejected, then confidence limits on the proportion of "foreign" animals in the marketplace can be determined, provided that the genetic composition of the source population of the "foreign" animals is known. (An example of this approach was given by Baker for North Pacific minke whales in 5.2, above.) Likewise, if the null hypothesis is not rejected, then the power of the data to detect various levels of admixture can be determined.

The question arose as to whether confidence limits or power can be determined when the identity or genetic composition of the alternative putative source population is unknown. Cooke suggested that a lower confidence bound on the admixture proportion (or, equivalently, an upper bound on the power to detect various levels of admixture) can be determined by assuming an extreme genetic composition of the alternative source. For example, if the market sample shows a lower occurrence of a given haplotype than the legitimate source population, then a lower confidence bound on the admixture of an alternative, as yet unidentified source population, can be computed by supposing that the occurrence of the given haplotype in the alternative source population is zero.

Recommendation 6: Improving Statistical Techniques. With regard to the need for better documentation of confidence in forensic results, the workshop recommended that, in addition to increasing samples in and improving access to global reference databases, old statistical techniques be tuned and new ones developed to address forensic questions specifically.

5.4. Individual Identification as an Alternative to Stock Identification

Continuing with discussions of the assignment test process, Palsbøll described his simulation analyses. Using a well-defined evolutionary model, these analyses examined how the ability to determine accurately population of origin is affected by (1) sample size, (2) genetic divergence between two hypothetical populations, and (3) number of tested loci. It has been argued in the literature that there is a diminishing return (in terms of correct assignment) with an increasing number of alleles per locus (e.g., Smouse and Chevillon, 1998). This observation was confirmed by Palsbøll's simulations.

In general, Palsbøll's simulations showed that the most appreciable increase in the ability to accurately assign population is obtained by increasing the number of loci, once sample sizes are above 50 individuals.

Although it was suggested that assignment testing provides a way of assigning individual test samples to place of origin without a decision about stock, it was also pointed out that *a priori* choices of reference populations are *de facto* stock decisions.

6. MARKET SURVEYS AND COLLECTION

In the following sections, market surveys of the sale of cetacean products for consumption in Japan, South Korea, Hong Kong, and Taiwan are described. Some case studies of other (non-cetacean) market surveys are also presented. The intention was to determine whether lessons could be drawn from other surveys with similar goals, i.e., to estimate the proportion of a certain product in the market. In addition, temporal changes in species compositions were examined over several published studies to evaluate the effects of different sampling strategies and collection teams.

6.1. Review of past Surveys

Japan

The workshop received descriptions of the sample collection methods employed in surveys by TRAFFIC, Earthtrust, IFAW, WDCS and Greenpeace. Results were also available from a survey by the JFA, but without information on how the survey had been designed. The results of all these surveys are summarized in Table 1.

The IFAW collections (items 3, 5, 7 and 10 in Table 1) conducted in Japan during 1995-99 were aimed mainly at covering a diversity of sources rather than proportional sampling. Shops potentially selling whale meat were identified from Yellow Pages, shopping guides and other sources. Samples have been obtained from 144 shops to date, covering about half the prefectures. A further 400-500 shops have been visited but no samples obtained. For each sample collected, photographs showing the type of product were archived, along with the date and place of purchase, nature of shop, and other information such as the label, advertisement, receipt, etc. and details about the block of tissue from which the sample was cut, where applicable (Funahashi and Mulvaney, 1998). Purchases tended to be targeted towards products that were unusual in one way or another, in order to minimize the probability that less common species would be missed by the survey. Since the surveys were aimed primarily at obtaining samples from baleen whales, products that appeared to be from toothed whales (e.g., dark-red meat, dark-tinted or thin blubber, and products with a distinctive toothed whale smell) were mostly avoided. The extent to which the selective sampling strategy is reflected in the results has not yet been specifically analyzed.

The Earthtrust and WDCS collections (items 3, 8, 11 in Table 1) were collected by agents from 1993-99. Care was taken to purchase products from a variety of outlets, including large department stores and smaller shops throughout Honshu and Kyushu, selected at random from available lists. At each outlet, a maximum of three samples differing in type and packaging were purchased but with no other regard to type or appearance. The sampling strategy was to limit collection of duplicate samples from the same individual whale and to enable reasonably unbiased estimation of overall market composition.

A survey conducted in Japan by TRAFFIC East Asia in April 1995 covered 904 retail outlets in 13 cities, of which 51 were found to be selling whale meat. A total of 53 whale meat samples were purchased (Chan *et al.*, 1995). Purchases focused on red meat and salted meat, but skin and blubber were also purchased if significant price differences were observed. The samples were analyzed by scientists at Hokkaido University in Japan commissioned by TRAFFIC

(using cytochrome *b* sequences) and again, independently, by the JFA (using control region sequences). There were some differences in the identifications (Phipps *et al.*, 1998) including: (1) southern minke vs. Dall's porpoise, which may have been related to mixing of the products at the market, cross-contamination at the marker, or the use of different markers by the two studies; and (2) northern vs. southern minke whales, which was probably related to the incomplete representation of minke whale diversity in the cytochrome *b* reference sequences. The workshop noted that additional documentation of the identification procedures followed would make it easier to determine the cause of the discrepancies.

Samples were also purchased in Japan in 1997 by an agent hired by Greenpeace Germany (Grohmann *et al.* in press); 44 samples were collected from 8 different cities throughout Japan; of these 38 were identified to species. The three samples identified as fin whale were found by control region analysis to have come from three different individuals, which were also distinct from the two fin whales sampled by Baker and Palumbi (1994). Two of the three were marketed as "fresh meat" but the possibility those had been frozen could not be excluded.

Repulic of Korea (South Korea)

Earthtrust (item 2 in Table 1) and IFAW (items 3 and 5 in Table 1) collections in co-operation with Greenpeace UK and the Korean Federation of Environmental Movements (KFEM) in South Korea during 1995-97 focused on products advertised as "large whale." Baleen whale products tended to be bought when these were visually identifiable; otherwise, frozen red meat, skin with blubber or mixed boiled organs were purchased.

TRAFFIC researchers surveyed whale meat outlets in Pusan and Ulsan in 1994-96, but did not purchase any samples, being under the impression that this would be illegal. Following clarification that disposal of bycatch products on local markets was permitted, 18 whale meat samples were purchased in 5 South Korean cities in April 1997. The samples were analyzed at the SWFSC. Many samples contained mixtures of species and the following species were identified: North Pacific minke whale, harbor porpoise (*Phocoena phocoena*), finless porpoise (*Neophocaena phocaenoides*), Risso's dolphin (*Grampus griseus*), Pacific white-sided dolphin (*Lagenorhynchus obliquidens*), false killer whale (*Pseudorca crassidens*), and short-beaked common dolphin.

Hong Kong

TRAFFIC surveyed 27 Japanese restaurants and 14 supermarkets and identified 7 restaurants who claimed they could supply whale meat. No samples were purchased since this is contrary to domestic law, but the Agriculture and Fisheries Department was informed. The department seized three suspected samples of which one was found to contain cetacean meat (short-finned pilot whale).

Taiwan

Trade in all cetacean products has been prohibited since 1990, with the exception of certain registered stockpiles that were exhausted by 1993. TRAFFIC researchers were unable to find any evidence of trade in large whale products although trade in small cetacean products is thought to be continuing.

6.2. Experience from Other Types of Market Surveys

Leaper presented case studies of market surveys from other sectors of industry. The intention was to identify similarities to the problem of estimating the proportions of different types of whale product on the market. However, none of the cases reviewed were sufficiently similar that the market survey techniques could be usefully applied to whale products. The conclusion was that estimating the proportions of whale products called for a unique approach. Leaper also discussed some aspects of data on market behavior that might be useful in designing surveys.

6.3. Combined Analysis of past Market Surveys for Whales

Cooke, Leaper and Lento presented a combined analysis of published results from the surveys of Japanese markets listed in Table A1 in Appendix 6. For the purpose of analysis, the data were summarized by year and survey, and the species were grouped into four categories: northern minke, southern minke, other baleen whales, and toothed whales. The results are shown in Appendix 6. A significant trend in grouped species composition over time was found but no significant differences were found in species composition between collection teams. This indicates that the representation of some species in some market surveys and not others can be explained in terms of random sampling variation. Hence, despite the differences in sampling strategies, it appears that all surveys conducted to date have yielded broadly similar results. Nevertheless, because of the difficulty of sampling the market uniformly, it cannot be assumed that the species occur in the market in the same proportions as that found in the surveys.

6.4. Implications for Future Market Surveys

There was a general discussion about the objectives of and requirements for potential market surveys. The following objectives were identified:

- Periodic general screening of markets to determine which species are in trade and, where possible, geographic origin of specimens in trade;
- Detection of violations (trade in products from illegal catches or imports), regardless of species;
- Estimation of takes from particular populations, such as the depleted J stock of minke whales that inhabits the Sea of Japan/East China Sea;
- Detection of trends in the species composition of the market;
- Detection of turnover rates and the number of individuals (and species) available in the market at any one time.

In view of the different purposes which market surveys can serve, no single sampling strategy can be specified as the best.

For the purpose of general screening, it is important that no major segments of the market in which products from particular species or sources might be sold, are under-represented. Knowledge of the overall structure and nature of the markets is helpful in ensuring that no major type of market is missed. Consumption of some species appeared to be concentrated in certain cities and regions, hence these species could be missed in a limited survey even if consumption is substantial. For example, the JFA 1995

survey, which did not cover Shizuoka prefecture, where most Dall's porpoises are consumed, yielded no Dall's porpoise samples, despite the high total national consumption of this species inferred from the level of national catch.

For detecting violations, it is necessary for legal and illegal products of the same species to be differentiated. Information should be available from every whale that has been legally caught or has legally entered the market through bycatch or stranding. In addition, information from previous legal catches that are still entering the market from stockpiles should be provided where this exists. It is impractical to sample from the current stockpiles themselves. The continued existence of legal stockpiles of undocumented composition remains a problem for the identification of illegal products in market samples. The workshop noted that it may be useful when collecting samples to examine them for signs of cell damage caused by freezing, in order to differentiate frozen products from fresh ones. However, once a product has been frozen it is probably impossible to determine how long it has been stored, because the decay rate depends on the state of freezing and the temperature and conditions under which it is kept frozen.

Recommendation 7: Mandatory Sampling of Whales that Enter Commerce. The workshop recommended that tissue samples from all whales destined for the marketplace, including those taken in whaling operations, as well as incidental takes and stranded animals, should be available for verification of specimen origin and for other management-related research. The same should apply to animals that were sampled when caught and whose products may still be in stockpiles.

A regulatory requirement that such samples be collected as a condition for being allowed to market the products from an incidentally caught or stranded individual might be an appropriate mechanism to ensure that this occurs.

For the purpose of estimating relative takes from specific populations, such as the J and O stocks of minke whales in the North Pacific, samples from each market segment need to be unselective, and may need to be scaled up by the relative size of each market segment in order to estimate the stock composition. The workshop recognized the difficulties of sampling products on the market in a strictly random fashion. It is difficult, if not impossible, to sample proportionally to the supply of each type of product. For example, the higher-quality cuts of meat may be available only in expensive restaurants and not appear in the retail markets.

Recommendation 8: Monitoring Markets that Sell Whale Products. The workshop recommended that monitoring of whale meat in the marketplace be continued and expanded to address a variety of issues, questions, and problems, including: providing information that could be relevant to implementation of the IWC's revised management scheme (RMS), for example on the occurrence in the marketplace of J and O stock minke whales from the western North Pacific; monitoring the appearance in the marketplace of rare or protected species; and identifying new conservation problems that might arise. The workshop emphasized that market sampling designs will vary, depending on the primary issue, question, or problem under investigation.

Recommendation 9: Specifications for Reporting on Species Identification. The

workshop recommended that procedures for determining species identities be reported explicitly, including: the genetic marker on which the species identification was based; primer sequences; decision criteria; and sources of reference sequences used for comparison (e.g., a GenBank accession number or, if unpublished, the researcher's name and contact details).

7. REFERENCE SAMPLES AND ARCHIVES

7.1. Review of Status of Reference Samples, Including Scientific Access

Chivers reported on tissue samples and genetic information held in the SWFSC collection. Currently, nearly 8000 tissue samples from cetaceans are included in the collection, from 73 of the 79 recognized species. The collection includes a number of samples from incidental fishery takes (30%) and biopsies (26%), plus a variety of other tissues from strandings, museums and other sources. Sixty-nine per cent of the samples were collected in U.S. national waters, the rest in international waters or numerous foreign locales. In total, 767 samples from baleen whales and 6887 samples from toothed whales are held (Table 2).

Requests for access to tissue samples are considered, contingent on their being used for specific studies rather than to build a general collection, the credentials of the person requesting samples, and availability of the sample types requested. Access to some specimens may be restricted at the request of the individuals who donated the samples, pending completion of their own studies and publication of results.

Yamada presented information on activities of the National Science Museum, Tokyo. The museum is currently involved in collection of tissue samples from strandings along the Japanese coast. In the past year, a genetics analysis laboratory has been established at the museum and a student has begun collection and genetic analysis of tissue samples. The number of animals processed by the stranding network has been increasing. In the first 6 months of 1999, necropsies of 30 strandings had been performed, including 2 minke whales and 28 toothed whales. Newly collected skeletal material prepared for the museum collection is not cleaned of attached soft tissue as carefully as in the past, since recent experience has shown that dried tissues can be a valuable source of material for genetic analysis. Many of the older cetacean skeletons in the museum collection, mostly from Japanese waters, include dried flipper tissues, which may also be useful for genetic analysis.

A number of universities, museums, aquariums, and the Institute for Cetacean Research (ICR) are involved in the stranding network, and large sections of coastline in Japan are covered. A newspaper clipping service helps to alert the network to published reports of strandings. In Japan, 9 baleen whale species and 37 toothed whale species were tabulated. Analysis of 64 stranding records from the last 10 years has revealed that Stejneger's beaked whale (*Mesoplodon stejnegeri)*, formerly thought rare, may actually be quite abundant in Japanese waters. Museum records from 1989 to 1999 showed that at least 4-20 minke whales were by-caught or stranded annually (Table 3). These could represent only a small percentage of the total incidental catch.

In response to questions, Yamada reported that necropsy analysis of stranded specimens has recently been instituted in order to obtain information about the cause of death in stranded animals. There was evidence from net marks on carcasses and "strandings" of severed heads that some stranded animals were actually derived from unreported directed or incidental take. In response to questions, Yamada said that minke whale strandings were not evenly distributed along the Japanese coast, and that a slightly higher proportion were reported from the west (Sea of Japan/East China Sea) coast. Between the museum and the ICR, skin samples for genetic research are collected from nearly all strandings. In addition to the 30-year-old specimens from J stock minke whales collected by Korean scientists and held by the ICR, additional samples from a number of stranded animals have been collected along the west coast of Japan by ICR personnel.

Dalebout summarized holdings in the New Zealand stranded cetacean tissue archive, housed at the School of Biological Sciences, University of Auckland (Table 2). Twenty-six to 28 cetacean species are found in New Zealand waters, including a great variety of beaked and baleen whale species. Tissue samples for genetic analysis are collected from all stranded cetaceans by Department of Conservation field agents. The tissue collection currently contains 287 cetacean samples, including 8 baleen whale species, 6 dolphin species, 9 beaked whale species, as well as sperm whales, pygmy sperm whales, and a spectacled porpoise (*Phocoena dioptrica*). Necropsies are performed on relatively fresh animals and each sample is accompanied by a stranding report, which contains the location and date of stranding and sample collection, external measurements, sex, and other information including the method of disposal. Field agents also submit copies of stranding reports for inclusion in the New Zealand Whale Stranding Database, housed and maintained at the Museum of New Zealand Te Papa Tongarewa, in Wellington.

This stranding database consists of over 2000 records for the New Zealand region and dates back more than 160 years. Pilot whales (*Globicephala* spp.) constitute the major portion of New Zealand strandings in terms of numbers of individuals, and tend to strand in large groups. Common dolphins are involved in the largest number of individual strandings, but fewer animals (mainly individuals) are involved per stranding event. Gray's beaked whale (*Mesoplodon grayi*) also strands relatively frequently along the New Zealand coast. In response to questions, Dalebout reported that there is a small amount of incidental catch involving mainly Hector's dolphins (*Cephalorhynchus hectori)* and dusky dolphins *(Lagenorhynchus obscurus)*, and this is mainly diagnosed from the presence of net marks on beachcast animals.

Lento reviewed contents and structure of the "Witness for the Whales" web site, which contains sequence information from cetacean products collected in Asian commercial markets (through 1997, at present). These sequences are freely available once potential users have applied for access through an e-mail registration process. The web site also contains a list of sequence identifiers used in various publications and reports. The first 70 base pairs are withheld from each sequence pending further analysis of haplotype frequencies and population identity, but enough sequence is available from each to confirm species identity. The web site database contains an additional set of approximately 20 reference sequences.

Daníelsdóttir summarized cetacean tissue

samples collected by the Marine Research Institute (MRI; Table 2), Iceland, and cooperative partners over the past 20 years. The collection contains many fin and sei whale samples and associated information on age, growth and reproduction. The largest part of the collection derives from the scientific whaling program carried out from 1986-1989. Genetic analysis performed on these samples focused on questions related to population structure. In addition, approximately 400 samples from minke whales were collected from 1980-1985. The collection also contains reference samples from four blue/fin hybrids collected in Icelandic waters in 1983, 1986, 1989 and 1998 (projectile biopsy). Another hybrid was provisionally identified on the basis of morphological characters shown in a photograph from 1972 (Árnason *et al.*, 1991; Árnason and Gullberg, 1993). Blue/fin hybrids have also been reported from Spain and the North Pacific, the latter based on morphological characters only (Bérubé and Aguilar, 1998). In response to a comment, Daníelsdóttir noted that new evidence suggests that the 1989 hybrid (a male) was fertile, in contrast to earlier reports.

A special program focused on incidental take between 1991-1997 involved collection of many harbor porpoises and white-beaked dolphins (*Lagenorhynchus albirostris)* and a few killer whale specimens. Fishermen were encouraged to return incidentally caught animals for analysis during this period, and received a cash payment for specimens provided. Return of incidentally caught specimens is still encouraged, but because of the difficulties involved, most bycatch is apparently discarded at sea.

Santos reviewed issues pertaining to conservation of marine species in the Philippines. Because of the high level of endemicity in this region, the Philippines is one of the highest-priority countries in the world for conservation research. Some 21-22 marine mammal species have been identified from the Philippines to date. Of particular interest are humpback whales (found in the northern region) and pygmy Bryde's whales. Sperm whale strandings have also been reported. All cetaceans are protected by national legislation (as are manta rays, giant clams, and whale sharks) and other marine species are regulated Tissue samples for genetic analysis have been archived in the tissue collection at the Southwest Fisheries Science Center (Table 2). This includes samples from several species of special interest such as pygmy or ordinary Bryde's whales (24 samples) and Fraser's dolphins (32 samples). Osteological material is additionally available at several museums and universities within the Philippines. Potential sources of additional tissues are strandings, meat sold in local markets, and biopsy sampling.

Perrin noted that a stranding program is being developed and that bycatch monitoring is part of a developing fishery monitoring program, but that little money is currently available for such programs. Santos added that an interagency network involving personnel in provincial offices has been instructed to collect tissue samples, but the stranding network is at an early stage. Some monitoring of fishery catches is carried out at the major landing sites.

In response to questions, Santos and Perrin explained that foreign vessels operating in Philippine waters do not carry observers at present, although such a program has been proposed. As a result, only anecdotal evidence is available regarding the fate of cetaceans taken incidentally by foreign fishing fleets, and the magnitude of the bycatch problem is hard to judge given the lack of observer data. Santos

noted that a large amount of fresh dugong meat cut into pieces was confiscated in 1995, possibly intended for export.

Palsbøll summarized the tissue samples in his collection at the University of Wales (Table 2) and the number of control region sequences and microsatellite profiles that have been analyzed for these samples to date. The purpose of this collection has been to characterize a few populations well, so tissues from a large number of individuals belonging to six North Atlantic species are included. A few samples from other oceans are also included for reference. The samples in Palsbøll's collection have been provided by a large number of collectors for collaborative research projects, and most of them cannot be shared except by permission of the original collector. Humpback whale samples and associated sequences are under control of the YONAH (Year of the North Atlantic Humpback) research group. Minke whale specimens in the collection were collected in the Gulf of Maine, Gulf of St. Lawrence, Greenland, Iceland, and Norway. In response to a question about the number of microsatellite loci that have been characterized, Palsbøll explained that 350 loci have been diagnosed, and primer sets for around 60 loci have been developed and are being used. Palsbøll also noted that he and Martine Bérubé are preparing papers on North Atlantic minke whales and participating in a worldwide study of minke whales headed by Luis Pastene (ICR). Thus associated genetic information should be available in the near future.

7.2. Procurement of, Archiving of, and Access to Current and Future Genetic Information

The workshop discussed the utility of genetic samples for a variety of research and management questions. It was agreed that tissue archives are an irreplaceable resource but that access to tissue samples was often difficult for a variety of reasons. Participants felt that the workshop could make useful suggestions about tissue collections, especially in the light of continuing discussions about such archives in the development of the RMS.

Recommendation 10: Long-term Tissue Sample Storage. Since tissue samples collected from cetaceans in most cases are extremely valuable or even irreplaceable and losses costly or catastrophic, the workshop recommended that such samples held for the purposes of research and management should be stored in duplicate and separate locations for the sake of long-term preservation.

Recommendation 11: Comprehensive Catalogue of DNA Tissue Samples. The workshop recommended that a comprehensive global catalogue of existing cetacean tissues held for genetic analysis be produced and updated on a regular basis.

The workshop was satisfied that the species identifications are reliable from the analyses that have been reported to date with details of the methods used. One caveat is that analyses using only mtDNA will have classified hybrids as the maternal species.

IWC resolution 1997-2 (see Appendix 2) encourages member countries to collect and inventory skin or meat samples for DNA identification from all whales that enter into commerce, which can include bycaught and stranded animals. Tissues being collected by IWC members now and during further development of the RMS could be valuable to a variety of future management issues, including

genetic tracking of market samples. Thus, the IWC may play an increasing role in holding or facilitating exchange of tissue archives necessary for its own research and management purposes.

Recommendation 12: Submission of Market Survey Results to the IWC Scientific Committee. The workshop concluded that within the context of the IWC, the Scientific Committee is the most appropriate forum for considering and evaluating the results of marketplace surveys of cetacean products. Therefore, the workshop recommended that such results be submitted directly to the Scientific Committee.

After discussion of the need for tissue archives to preserve samples from cetaceans available from a variety of sources, the workshop considered the need for accessibility to genetic information from such specimens. Access to such data is needed for various management and research purposes, but this information is often held by researchers unwilling to release it until particular research programs are completed and published. Genetic profiles are also being collected from individual whales taken in scientific and commercial hunts, and these are held in proprietary databases by individual countries. IWC resolution 1997-2 encourages contracting governments to make such genetic databases available. An additional consideration is the increasing size of databases being compiled by large research programs and national fishery agencies, making it harder to manage access to and distribution of such information. Large public sequence databases such as GenBank were not designed for documenting intra-specific variability from a large number of individuals, although such information is collected in an increasing number of population genetic studies.

Recommendation 13: Controlled Access to Genetic Databases. The workshop recommended that a verifiable mechanism be developed to allow controlled access to genetic databases compiled for research and management purposes from scientific and commercial catches, biopsy programs, stranding networks and museum collections.

Given that valuable genetic information is held both in private and freely-accessible public databases, such an access mechanism should, for example, allow searching for matches to market samples without release of data held in private research archives and national commercial catch databases. If this sort of mechanism were developed, individual researchers and fishery agencies could allow access to unpublished and proprietary information without release of all of the original sequence data or microsatellite profiles.

Dizon suggested that an Internet-based search engine, such as implemented in the GenBank BLAST search function, provides a model for such a method that could restrict release of proprietary information but allow detection of matches from market samples, monitored bycatch, and stranded individuals.

Recommendation 14: Design Specification of Controlled Access Databases. As a first step in the implementation of a controlled access database, the workshop recommended that a group of interested and qualified scientists be convened to establish the design specifications. The design specifications should cover aspects such as database structure and format, user interface, verification and security safeguards. Safeguards must prevent unauthorized access to the database itself and control the

information returned that is sent in response to queries. The owners of private individual or proprietary national sequence databases must be confident that their interests will be protected.

LITERATURE CITED

Árnason, Ú., and Gullberg, A., 1993. Comparison between the complete mtDNA sequences of the blue and fin whale, two species that can hybridize in nature. *Journal of Molecular Evolution* 37:312-322.

Árnason, Ú., Spilliaert, R., Palsdottir, A., and Árnason, A., 1991. Molecular identification of hybrids between the two largest whale species, the blue (*Balaenoptera musculus*) and the fin (*B. physalus*). *Hereditas* 115:183-198.

Baker, C. S., and Palumbi, S. R., 1994. Which whales are hunted? A molecular genetic approach to monitoring whaling. *Science* 265:1538-1539.

Baker, C. S., Cipriano, F., and Palumbi, S. R., 1996a. Molecular genetic identification of whale and dolphin products from commercial markets in Korea and Japan. *Molecular Ecology* 5:671-685.

Baker, C. S., Cipriano, F., Lento, G. M., and Palumbi, S. R., 1996b. Whale and dolphin products for sale in Japan and Korea, 1993-95. Unpublished report to the Scientific Committee of the International Whaling Commission (SC/48/O38).

Baker, C. S., Dalebout, M. L., Congdon, B. C., and Lento, G. M., 1999. Molecular genetic identification of whale and dolphin products for sale in Japan, 1998-99. Unpublished report to the Scientific Committee of the International Whaling Commission (SC/51/O15).

Baron, H., Fung, S., Aydin, A., Bähring, S., Luft, F. C., and Schuster, H., 1996. Oligonucleotide ligation assay (OLA) for the diagnosis of familial hypercholesterolemia. *Nature Biotechnology* 14:1279-1282.

Bérubé, M., and Aguilar, A., 1998. A new hybrid between a blue whale, *Balaenoptera musculus*, and a fin whale, *B. physalus*: Frequency and implications of hybridization. *Marine Mammal Science* 14:82-98.

Bowen, B. W., and Avise, J. C., 1994. Conservation research and the legal status of PCR products. *Science* 266:713.

Chan, S., Ishihara, A., Lu, D. J., Phipps, M., and Mills, J. A., 1995. Observations on the whale meat trade in East Asia. *TRAFFIC Bulletin* 15:107-115.

Cipriano, F., and Palumbi, S. R., 1999a. Genetic tracking of a protected whale. *Nature* 397:307-308.

Cipriano, F., and Palumbi, S. R., 1999b. Rapid genotyping techniques for identification of species and stock identity in fresh, frozen, cooked, and canned whale products. Unpublished report to the Scientific Committee of the International Whaling Commission (SC/51/O9).

Cipriano, F., and Palumbi, S. R., 1997. DNA forensics and population structure in North Pacific minke whales. Unpublished report to the Scientific Committee of the International Whaling Commission (SC/49/NP17).

Congdon, D. C., Lento, G. M., and Baker, C. S., 1999. High-resolution mtDNA identification of minke whale stocks in the North Pacific. Unpublished report to the Scientific Committee of the International Whaling Commission (SC/51/RMP20).

Dalebout, M. L., Van Helden, A., Van Waerebeek, K., and Baker, C. S., 1998. Molecular genetic identification of southern hemisphere beaked whales (Cetacea: Ziphiidae). *Molecular Ecology* 7:687-694.

DeSalle, R., and Birstein,V. J., 1996. PCR identification of black caviar. *Nature* 381:197-198.

Dietmaier, W., Hartmann, A., Wallinger, S., Heinmöller, E., Kerner, T., Endl, E., Jauch, K.-W., Hofstädter, F., and Rüschoff, J., 1999. Multiple mutation analyses in single tumor cells with improved whole genome amplification. *American Journal of Pathology* 154:83-95.

Dizon, A. E., Chivers, S. J., and Perrin, W. F. (eds.) 1997. *Molecular Genetics of Marine Mammals.* Special Publication Number 3, The Society for Marine Mammalogy. Lawrence, Kansas, USA.

Funahashi, N., and Mulvaney, K. (eds.), 1998. *Whale for sale: illegal trade in whale meat and products in Japan and South Korea*. International Fund for Animal Welfare, P.O. Box 193, Yarmouth Port, MA USA.

Goto, M., and Pastene, L. A., 1997. Population structure in the western North Pacific minke whale based on an RFLP analysis of the mtDNA control region. *Report of the International Whaling Commission* 47:531-538.

Government of Japan, Fisheries Agency., 1997. Results of molecular genetic analysis of whale products collected from Japanese retail markets in 1995. Unpublished report to the Infractions Committee of the International Whaling Commission (IWC/49/INF3).

Grohmann, L., Bokerman, I., Sonntag, R., Unseld, M., Hiesel, R., Malek, O., Giese, A., and Brennicke, A., In press. Whale meat from protected species is still being sold on Japanese markets. *Naturwissenschaften*

Henshaw, M. D., LeDuc, R. G., Chivers, S. J., and Dizon, A. E., 1997. Identification of beaked whales (family Ziphiidae) using mtDNA sequences. *Marine Mammal Science* 13:487-495.

Hobbs, R. C., Rugh, D. J., and DeMaster, D. P., 1999. Abundance of beluga whales in Cook Inlet, Alaska, 1994-1998. Unpublished report to the Scientific Committee of the International Whaling Commission (SC/51/SM8).

Jones, M., 1994. PCR products and CITES. *Science*, 266:1930.

Lento, G. M., Patenaude, N. J., and Baker, C. S., 1997. Molecular genetic identification of whale and dolphin products for sale in Japan and Korea, 1995-97. Unpublished report to the Scientific Committee of the International Whaling Commission (SC/49/O21).

Lento, G. M., Dalebout, M. L., and Baker, C. S., 1998a. Species and individual identification of whale and dolphin products for sale in Japan by mtDNA sequences and nuclear microsatellite profiles. Unpublished report to the Scientific Committee of the International Whaling Commission (SC/50/08).

Lento, G. M., Cipriano, F., Patenaude, N. J., Palumbi, S. R, and Baker, C. S., 1998b. Taking stock of minke whales in the North Pacific: origins of products for sale in Japan and Korea. Unpublished report to Scientific Committee of the International Whaling Commission (SC/50/RMP15).

Lopez, J. V., Yuhki, N., Masuda, R., Modi, W., and O'Brien, S. J., 1994. Numt, a recent transfer and tandem amplification of mitochondrial DNA to the nuclear genome of the domestic cat. *Journal of Molecular Evolution* 39: 174-190.

Malik, S., Wilson, P. J., Smith, R. J., Lavigne, D. M., and White, B. N., 1997. Pinniped penises in trade: a molecular genetic investigation. *Conservation Biology* 11:1365-1374.

Mullis, K. B., and Faloona, F. A., 1987. Specific synthesis of DNA *in vitro* via a polymerase-catalyzed chain reaction. *Methods in Enzymology* 155:335-350.

O'Corry-Crowe, G. M., Suydam, R. S., Rosenberg, A., Frost, K. J., and Dizon, A. E., 1997. Phylogeography, population structure and dispersal patterns of the beluga whale *Delphinapterus leucas* in the western Nearctic revealed by mitochondrial DNA. *Molecular Ecology* 6:955-970.

Palumbi, S. R., and Baker, C. S., 1994. Contrasting population structure from nuclear intron sequences and mtDNA of humpback whales. *Molecular Biology and Evolution* 11:426-435.

Pella, J. J., and Milner, G. B., 1987. Use of genetic markers in stock composition analysis. In N. Ryman and F. Utter (eds.), *Population Genetics and Fisheries Management*, pp. 247-276. University of Washington Press. Seattle, Washington, USA.

Phipps, M., Ishihara, A., Kanda, N., and Suzuki, H., 1998. A preliminary report on DNA sequence analysis of whale meat and whale meat products collected in Japan. *TRAFFIC Bulletin* 17:91-94.

Rice, D. W., 1998. *Marine Mammals of the World. Systematics and Distribution*. Special Publication Number 4, *The Society for Marine Mammalogy*. Lawrence, Kansas, USA.

Roman, J., and Bowen, B. W. in press. Mock turtle syndrome: genetic identification of turtle meat purchases in the southeast United States. *Animal Conservation*.

Rosel, P. E., Dizon, A. E., and Heyning, J. E., 1994. Genetic analysis of sympatric morphotypes of common dolphins (genus: *Delphinus*). *Marine Biology* 119: 159-167.

Smouse, P.E., and Chevillon, C., 1998. Analytical aspects of population-specific DNA fingerprinting for individuals. *Journal of Heredity* 89:143-150.

Taylor, B. L., 1997. Defining "population" to meet management objectives for marine mammals. *In* A. E. Dizon, S.J. Chivers and W.F. Perrin (eds.) *Molecular Genetics of Marine Mammals*. Special Publication Number 3, *The Society for Marine Mammalogy*. Lawrence, Kansas, USA. pp.46-66.

Taylor, B. L., and Dizon, A. E., In press. First policy then science: Why a management unit based solely on genetic criteria can't work. *Molecular Ecology*.

Table 1. A summary of the molecular genetic identification of whales, dolphins and porpoises sold in commercial markets of Japan (J) and the Republic of Korea (K), including the reference for the report (number 1-11) and the organization or agency supporting the survey (ET, Earthtrust; GPG, Greenpeace Germany; IFAW, International Fund for Animal Welfare; JFA, Fisheries Agency of Japan; TRAF, TRAFFIC; Pew, Pew Charitable Trusts; WDCS, Whale and Dolphin Conservation Society). 1. Baker and Palumbi, 1994; 2. Baker *et al.*, 1996a; 3. Baker *et al.*, 1996b; 4. JFA, 1997; 5. Lento *et al.*, 1997; 6. Phipps *et al.*, 1998; 7. Lento *et al.*, 1998a; 8. Cipriano and Palumbi, 1997; 9. Grohman *et al.*, in press; 10. Baker *et al.*, 1999; 11. Cipriano and Palumbi, 1999b.

Location	K	K	K	Korea	J	J	J	J	J	J	J	J	J	J	Japan	Species
Organization	ET	IFAW/ ET	IFAW	Totals	ET	IFAW/ ET	IFAW	JFA	TRAF	IFAW	ET	GPG	IFAW	Pew/ WDCS	Totals	Totals
Report no.	2	3	5		1	3	5	4	6	7	8	9	10	11		
N. Minke	13	13	28	54	1	6	9	12	2	15	9	8	29	29	120	174
S. Minke		2		2	8	51	36	112	31	39	46	22	57	56	458	460
Dwarf Minke								1							1	1
Bryde's		2		2		2	2		1					1	6	8
Pygmy Bryde's	2			2											0	2
Sei										1			4		5	5
Humpback					1		1							2	4	4
Fin					4	6	7	15	2	4	2	3	1		44	44
Blue or fin/blue [1]						2									2	2
Sperm[2]								2					1	2	5	5
Pygmy Sperm										1					1	1
Baird's Beaked						10	4	14	2	5	5	1	8	7	56	56
Cuvier's Beaked	1			1		1	2		1		1				6	6
Other Beaked							1	3				1			5	5
Porpoise						1	1		6	1	6	1	2	1	19	19
Killer whale			1	1			1								1	2
Dolphins	1	13	5	19	3	7	12	1	4	8	18	2	15	15	85	104
Artiodactyl													1		1	1
Sheep							2								2	2
Horse													2		2	2
Unidentifiable								15	4		9	6		17	51	51
Total	17	30	34	81	17	86	78	175	53	74	96	44	120	130	873	954

[1] Entry includes an animal established to be a hybrid between a fin and blue whale. It also includes an animal identified as a blue whale with mtDNA, which does not rule out the possibility that it is a fin male / blue female hybrid.

[2] Entry for 2 sperm whales in JFA report is not specific. JFA reports only "two spp of Physeterids."

Table 2. Summary of specimens in four molecular genetics tissue/DNA archives reviewed during the Workshop. SWFSC = Southwest Fisheries Science Center, USA (samples from Philippines after comma); AUNZ = Auckland University, NZ; MRI = Marine Research Institute, Iceland; UW = University of Wales, UK. Includes specimens referred to species either morphologically or genetically. Taxonomic usage follows Rice (1998). Notation + = present, but numbers not given. [1] Some entries in the ziphiid section of this database are duplicates of material held at SFWC and vice versa and does not include tissue from biopsy sampling. [2] nomenclature uncertain. [3] identification uncertain for three samples (see Dalebout *et al.*, 1998; Henshaw *et al.*, 1997).

		SWFSC	AUNZ[1]	MRI	UW
BALEEN WHALES					
Balaenopteridae					
blue whale	*Balaenoptera musculus*	303	28	+	161
fin whale	*B. physalus*	36	21	+	661
pygmy & ordinary Bryde's whale	*B. edeni/brydei*[2]	73,24	10		
sei whale	*B. borealis*	11	1	+	
minke whale	*B. acutorostrata*	22	1	+	437
Antarctic minke whale	*B. bonaerensis*		1		
humpback whale	*Megaptera novaeangliae*	110	560	+	3729
Eschrichtiidae					
gray whale	*Eschrichtius robustus*	124	12		
Balaenidae					
bowhead whale	*Balaena mysticetus*	65			
right whale	*B. glacialis*	11	241		
Neobalaenidae					
pygmy right whale	*Caperea marginata*	4	6		
TOOTHED WHALES					
Ziphidae					
Baird's beaked whale	*Berardius bairdii*	6			
Arnoux's beaked whale	*B. arnuxii*	1	1		
Sowerby's beaked whale	*Mesoplodon bidens*	12			
Blainville's beaked whale	*M. densirostris*	3	3	+	
Gervais' beaked whale	*M. europaeus*	10			
Stejneger's beaked whale	*M. stejnegeri*	15			
Hubbs' beaked whale	*M. carlhubbsi*	9			
Hector's beaked whale	*M. hectori*	43	2		
True's beaked whale	*M. mirus*	4			

		SWFSC	AUNZ[1]	MRI	UW
strap-toothed whale	*M. layardii*	4	10		
Gray's beaked whale	*M. grayi*	5	58		
Andrews' beaked whale	*M. bowdoini*	1	2		
gingko-toothed whale	*M. ginkgodens*	1			
Sheperd's beaked whale	*Tasmacetus shepherdi*	6	4		
northern bottlenose whale	*Hyperoodon ampullatus*	1		+	
southern bottlenose whale	*H. planifrons*	2	5		
Cuvier's beaked whale	*Ziphius cavirostris*	30,1	9	+	
unknown beaked whale	*M. sp.*	5			
Physeteridae					
sperm whale	*Physeter macrocephalus*	331,2	18	+	
pygmy sperm whale	*Kogia breviceps*	74	25		
dwarf sperm whale	*K. sima*	34,3			
Iniidae					
botu	*Inia geoffrensis*	4			
Potoporiidae					
Franciscana	*Pontoporia blainvillei*	30			
Monodontidae					
beluga	*Delphinapterus leucas*	962			1271
narwhal	*Monodon monoceros*	92			617
Delphinidae					
short-beaked common dolphin	*Delphinus delphis*	743	13		
long-beaked common dolphin	*D. capensis*	132			
unknown common dolphin	*Delphinus sp.*	8			
spotted dolphin	*Stenella attenuata*	769,17			
Atlantic spotted dolphin	*S. frontalis*	50			
spinner dolphin	*S. longirostris*	460,106			
striped dolphin	*S. coeruleoalba*	486		+	
clymene dolphin	*S. clymene*	32			
unknown spotted dolphin	*Stenella sp.*	7			
rough-toothed dolphin	*Steno bredanensis*	29			
bottlenose dolphin	*Tursiops truncatus/aduncus*	1032,4	16		
Pacific white-sided dolphin	*Lagenorhynchus obliquidens*	169			
white-beaked dolphin	*L. albirostris*	5		+	
Atlantic white-sided dolphin	*L. acutus*	24		+	
dusky dolphin	*L. obscurus*	2	12		

		SWFSC	AUNZ[1]	MRI	UW
hourglass dolphin	*L. cruciger*	3			
Peale's dolphin	*L. australis*	4			
northern right whale dolphin	*Lissodelphis borealis*	134			
southern right whale dolphin	*L. peronii*	2			
Fraser's dolphin	*Lagenodelphis hosei*	45,32			
Commerson's dolphin	*Cephalorhynchus commersonii*	2			
black dolphin	*C. eutropia*	1			
Hector's dolphin	*C. hectori*	1	30		
Heaviside's dolphin	*C. heavisidii*	1			
Irrawaddy dolphin	*Orcaella brevirostris*	14			
Indo-Pacific humpbacked dolphin	*Sousa plumbea/chinensis*	37			
tucuxi	*Sotalia fluviatilis*	2			
Risso's dolphin	*Grampus griseus*	55,1			
short-finned pilot whale	*Globicephala macrorhynchus*	114			
long-finned pilot whale	*G. melas*	118		+	
unknown pilot whale	*Globicephala sp.*	18	49		
melon-headed whale	*Peponocephala electra*	3,1			
false killer whale	*Pseudorca crassidens*	15			
pygmy killer whale	*Feresa attenuata*	13,2			
killer whale	*Orcinus orca*	18	6	+	
Phocoenidae					
harbor porpoise	*Phocoena phocoena*	304		+	
vaquita	*P. sinus*	50			
Burmeister's porpoise	*P. spinipinnis*	4			
spectacled porpoise	*P. dioptrica*	4	1		
finless porpoise	*Neophocaena phocaeoides*	56			
Dall's porpoise	*Phocoenoides dalli*	261			

Table 3. Reported stranded and incidentally caught North Pacific minke whales recorded by the National Science Museum, Tokyo. (Note that only the first six months of 1999 included.)

Year	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999
Number	17	7	4	4	10	10	12	22	20	14	9

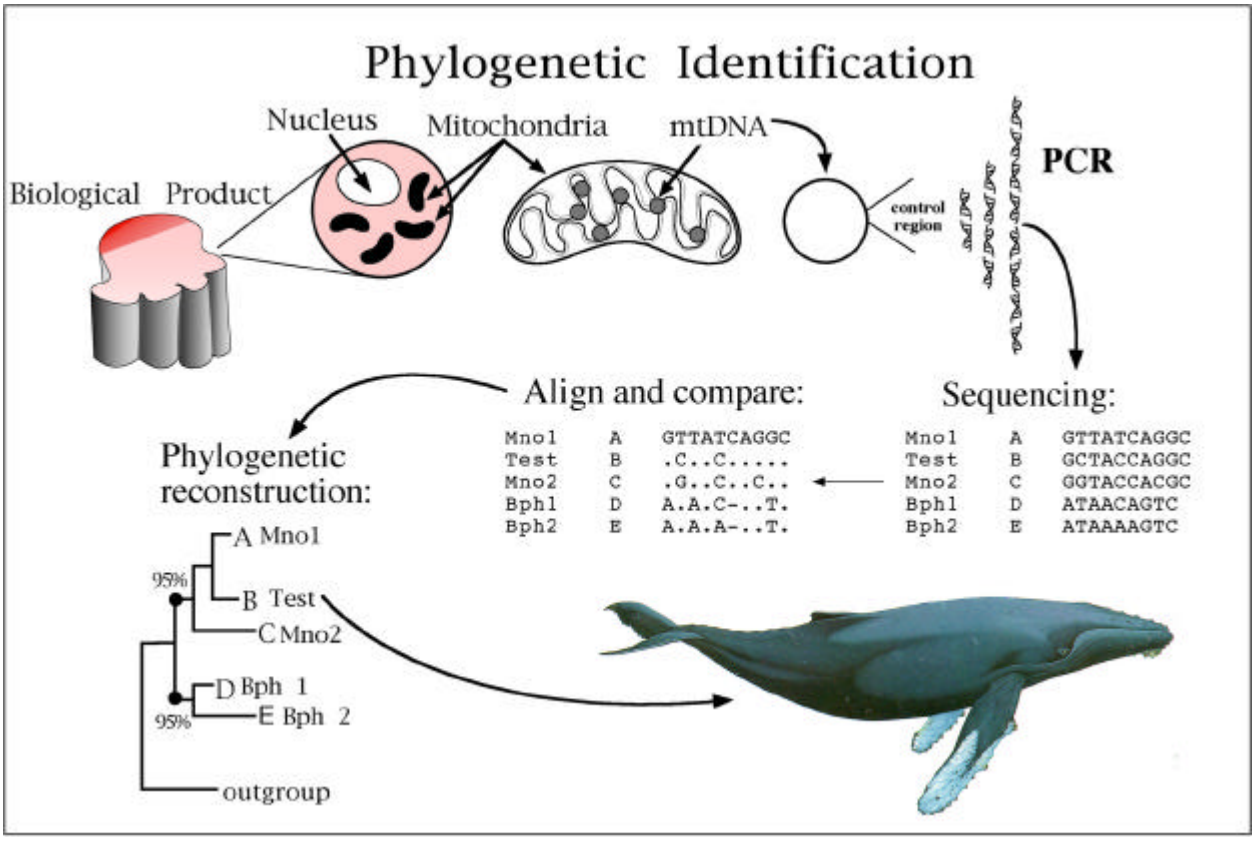

Figure 1. The basic steps involved in the phylogenetic identification of an unknown specimen or biological product. First, mtDNA is extracted from the product in question. Second, a fragment of the mtDNA, e.g., control region, is amplified from the product via PCR (usually less than 1,000 base pairs). Third, the exact nucleotide sequence of the amplified fragment is determined by automated electrophoresis. Fourth, the sequence of the product, now referred to as the "test," is aligned and compared with the sequences from reference samples. Finally, the sequence from the product is grouped, by phylogenetic reconstruction, with the most closely related reference sequences. The reconstruction is usually represented as a "tree," with closely related sequences forming neighboring branches. This allows an hierarchical comparison to establish, first, the suborder and family derivation using a small number of reference sequences from a large number of species. A close relation, or match with a reference sequence provides evidence for identification of the species origin of the product. One or more "outgroups" (i.e., distantly related species) are used to protect against misclassification error. Re-sampling procedures are used to indicate the relative degree of reliability or consistency of groupings among reference and test sequences.

Appendix 1. List of participants (Asterisks [*] denote members of the Workshop Steering Committee).

Scott Baker *
School of Biological Sciences
Auckland University
Pvt Bag 92019
Auckland, New Zealand
Tel: 64-9-373-7599 ext. 7280
Fax: 64-9-373-7599 ext. 7417
Email: cs.baker@auckland.ac.nz

Brian Bowen
University of Florida
Department of Fisheries Science
& Aquatic Sciences
7922 NW 71st Street
Gainesville, Fl 32653, USA
Tel: 1-352-392-9617 ext. 280
Fax: 1-352-846-1088
Email: bowen@gnv.ifas.ufl.edu

Robert Brownell *
Southwest Fisheries Science Center
National Marine Fisheries Service
P.O. Box 271
La Jolla, CA 92038, USA
Tel: 1-619-546-7165
Fax: 1-619-546-5653
Email: brownell@caliban.ucsd.edu

Frank Cipriano
Department of Biology
San Francisco State University
1600 Holloway Ave.
San Francisco, CA 94132
Tel: 1-415-338-7818
Fax: 1-415-338-6245
Email: cipriano@sfsu.edu

Justin Cooke
CEMS
Mooshof
79297 Winden, Germany
Tel: 49-7681-6018
Fax: 49-7681-6019
Email: jgc@cems.de

Merel Dalebout
School of Biological Sciences
Auckland University
Pvt Bag 92019
Auckland, New Zealand
Tel: 64-9-373-7599 ext. 4588
Fax: 64-9-373-7599 ext. 7417
Email: m.dalebout@auckland.ac.nz

Anna Daníelsdóttir
The Population Genetic Laboratory
Marine Research Institute
c/o Biotechnology House
Keldnaholt, IS-112
Reykjavik, Iceland
Tel: 354-570-7217
Fax: 354-570-7111
Email: andan@iti.is

Andrew Dizon *
Southwest Fisheries Science Center
National Marine Fisheries Service
P.O. Box 271
La Jolla, CA 92038, USA
Tel: 1-619-546-7089
Fax: 1-619-546-7003
Email: adizon@ucsd.edu

Naoko Funahashi *
IFAW
3-7-9-210 Shimohoya, Hoya-shi,
Tokyo 202-0004, Japan
Tel: 81-424-23-8779
Fax: 81-424-23-8779
Email: funahas@ibm.net

Lutz Grohmann
BioInside GmbH
Warthestr. 21
D-14513 Teltow bei Berlin, Germany
Tel: 49-3328-205190
Fax: 49-3328-305192
Email: grohmann@MPIMG-Berlin-Dahlem.MPG.DE

Toshio Kasuya
Seibutsushigen Gakubu
Mie University
1515 Kamihama-cho
Tsu, Mie, 514-8507, Japan
Tel: 81-59-231-9543
Fax: 81-59-231-9538
Email: kasuya@bio.mie-u.ac.jp

Jae-Heup Kim
Laboratory of Genomic Diversity
National Cancer Institute
Bldg 560, Rm 11-12
Frederick, MD 21702-1201, USA
Tel: 1-301-846-1299
Fax: 1-301-846-1909
Email: kimjae@mail.ncifcrf.gov

David Lavigne
International Marine Mammal Assoc.
1474 Gordon Street
Guelph, Ontario N1L 1C8, Canada
Tel: 1-519-767-2548
Fax: 1-519-767-0284
Email: dlavigne@imma.org

Russell Leaper
30 Ivy Terrace
Edinburgh, EH11 1PJ, Scotland, UK
Tel: 44-131-346-7461
Fax: 44-131-337-9640
Email: 0002054254@mcimail.com

Gina Lento *
School of Biological Sciences
Auckland University
Pvt Bag 92019
Auckland, New Zealand
Tel: 64-9-373-7599 ext. 7217
Fax: 64-9-373-7599 ext. 7417
Email: g.lento@auckland.ac.nz

Per Palsbøll
School of Biological Sciences
University of Wales
Deiniol Road, Bangor
Gwynedd LL57 2UW, Wales, UK
Fax: 44-1248-372825
Email: p.palsboll@bangor.ac.uk

Vassili Papastavrou *
IFAW
17 Hartington Park,
Bristol BS6 7ES, England, UK
Tel: 44-1179-249109
Fax: 44-1179-445263
Email: vpapastavrou@ifaw.org

William Perrin
Southwest Fisheries Science Center
National Marine Fisheries Service
P.O. Box 271
La Jolla, CA 92038, USA
Tel: 1-619-546-7096
Fax: 1-619-546-7003
Email: wperrin@ucsd.edu

Marcus Phipps
Room 2001, Double Bldg.
22 Stanley Street
Central, Hong Kong
Tel: Direct: 852-2973 9495
Fax: 852-2530 0864
Email: mphipps@asiaonline.net

Randall Reeves (Chair) *
27 Chandler Lane
Hudson, Quebec
J0P 1H0, Canada
Tel: 1-450-458-6685
Fax: 1-450-458-7383
Email: rrreeves@total.net

Mudjekeewis D. Santos
Bureau of Fisheries and Aquatic Resources
Department of Agriculture
860 Acradia Bldg., Quezon Ave.
Quezon City
Philippines
Tel: 63-2-373-7451
Fax: 63-2-373 -7449
Email: mudjie@mnl.v-link.net

Barbara Taylor
Southwest Fisheries Science Center
National Marine Fisheries Service
P.O. Box 271
La Jolla, CA 92038
Tel: 1-619-546-5620
Fax: 1-619-546-7003
Email: taylor@caliban.ucsd.edu

Tadasu Yamada
Department of Zoology
National Science Museum, Tokyo
3-23-1 Hyakunin-cho
Shinjuku-ku, Tokyo 169-0073, Japan
Tel: 81-3-3364-2311 x7168
Fax: 81-3-3364-7104
Email: yamada@kahaku.go.jp

Observers:

Southwest Fisheries Science Center

Susan Chivers, Peter Dutton, Rick LeDuc,
Greg O'Corry-Crowe
National Marine Fisheries Service
P.O. Box 271
La Jolla, CA 92038, USA
Tel: 1-619-546-7000
Fax: 1-619-546-7003
Email: schivers@ucsd.edu,
peterd@caliban.ucsd.edu,
rleduc@ucsd.edu,
gocrowe@caliban.ucsd.edu

World Wildlife Fund

Karen Baragona
WWF
1250 24th Street NW
Washington, D.C. 20037
Tel: 1-202-778-9674
Fax: 1-202-293-9345
Email: Karen.Baragona@WWFUS.ORG

Appendix 2. IWC resolutions 1997-2 and 1999-8.

CHAIRMAN'S REPORT OF THE FORTY-NINTH ANNUAL MEETING
IWC Resolution 1997-2
RESOLUTION ON IMPROVED MONITORING OF WHALE PRODUCT STOCKPILES

RECOGNISING the progress in establishing reliable techniques for identifying the origin of whale meat and whale products, including the species and geographic stock of origin and individual identification of legally obtained and marketed whale products, through DNA testing and genetic analysis;

NOTING the recent accomplishments of Japan, Norway and the United States in the establishment of reference sets of 'type species' of cetacean DNA sequences for use in addressing the problems of unreported bycatch and illegal trade by determining the source species and geographic origin of such products and the development of market survey programmes utilising DNA testing by some member governments;

RECOGNISING that some whale products legally sold in the domestic markets of some countries are from sources (such as frozen stockpiles and fisheries bycatch) that are not systematically sampled, making it difficult for fisheries personnel to develop market survey programmes to determine the origin of whale meat sold commercially; RECOGNISING FURTHER that CITES has called upon member nations to report on the status of stockpiles of whale meat, in order to facilitate the monitoring of illegal trade, and has invited all countries concerned to cooperate in determining the sources of whale meat in cases of smuggling or unknown identity;

NOW THEREFORE the Commission:

ENCOURAGES all Contracting Governments to provide information to the IWC about the size of remaining stockpiles and the species of origin of meat remaining in stockpiles, and to collect and inventory skin or meat samples for DNA identification from all whales that enter into commerce, and to make the DNA database available to the IWC;

REQUESTS that the IWC Secretariat forward to the CITES Secretariat this Resolution and this year's reports of the infractions Sub-committee and the Scientific Committee.

CHAIRMAN'S REPORT OF THE FIFTY-FIRST ANNUAL MEETING
IWC Resolution 1999-8
RESOLUTION ON DNA TESTING

RECALLING THAT the Commission is developing a Revised Management Scheme that will require regular updates on relevant new methods and technologies for the inspection and monitoring of commercial whaling operations;

NOTING THAT one of the most promising of these technologies is DNA-based identification of market products and genetic typing of known catches;

The Commission now therefore:

REQUESTS the Scientific Committee to establish an agenda item to provide annual reports on progress in the following areas:

a) Genetic methods for species, stock and

individual identification;

b) Collection and archiving of tissue samples from catches and by-catch;

c) Status of and conditions for access to reference databases of DNA sequences or microsatellite profiles derived from directed catches, by-catch, frozen stockpiles and products impounded or seized because of suspected infractions.

AND FURTHER REQUESTS the Scientific Committee to provide advice to the Commission on the development and implementation of a transparent and verifiable system of identification and tracking of products derived from whales taken under the RMP, and to provide a means to differentiate such products from those taken outside the RMP.

Appendix 3. Agenda of the workshop. Included (in italics) are the authors and titles of working papers presented during the meeting. Drafts of these papers may be available from the authors at their discretion.

1. Introduction.
   1.1. Opening of the workshop.
   1.2. Appointment of rapporteurs.
   1.3. Chairman's remarks.
   1.4. Adoption of agenda.

2. Overview of Molecular Identification of Cetaceans: Selected Case Studies
   2.1. Baleen whales in commercial markets
      C. S. Baker and G. M. Lento. Molecular monitoring of baleen whales on commercial markets: an overview and prospectus.
   2.2. Toothed whales in by-catch
      S. J. Chivers and K. M. Robertson. Confirmation of odontocete field identifications made by observers in the California gillnet fisheries.
   2.3. Beaked whale strandings
      M. L. Dalebout and C. S. Baker. Molecular genetic identification of stranded beaked whales (Ziphiidae) in New Zealand.

3. Lessons from Molecular Monitoring in Other Taxa
   3.1. Turtles
      B. W. Bowen. Molecular monitoring in other species: Turtles.
   3.2. Sturgeon
   3.3. Pinnipeds
      D. M. Lavigne, P. J. Wilson, R. J. Smith, and B. N. White. Pinniped penises in the marketplace: a progress report.

4. Methods for Molecular Identification of Species
   4.1. Phylogenetic methods, confidence and consistency of identification
      G. M. Lento and F. Cipriano. Phylogenetic identification methods, statistical confidence and consistency of species identification.
      J. -H. Kim. Mitochondrial DNA variations, NuMt and heteroplasmy, in animals.
   4.2. Comparative power of molecular markers and adequacy of reference databases
      A. E. Dizon, A. Frey, A. Rosenberg, and R. LeDuc. Intra- and inter-specific variability and the species identification process.
      M. L. Dalebout. Comparative consistency and sensitivity of the mtDNA control region and cytochrome b *for species identification of beaked whales (Ziphiidae).*
      Summaries of reference databases:
      - The Baker *et al.* reference database
      - The cetacean control region identification database used at the Center for Conservation and Evolutionary Genetics at Harvard University
      - The SWFSC reference database (Table 1.Item 4.2.1)
      - The Japan Fisheries Agency reference database (IWC/49/INF3)

4.3. Technical limitations and advances

F. Cipriano and S. R. Palumbi. Technical advances in genotyping techniques for identification of species and stock identity of whale products.

G.M. Lento and M.L. Dalebout. Species-specific PCR for field-based species identification screening.

5. Genetic Identification of Geographic Origin and "Stock" Identity
   5.1. Stock definitions under various management schemes
   5.2. Phylogenetic and statistical methods for population analysis

C. S. Baker, G. M. Lento, F. Cipriano, S. R. Palumbi, and B. C. Congdon. North Pacific minke whales on the Japanese markets: species identification, stock estimation and individual exclusion.

G. M. O'Corry-Crowe and L. S. Lowry. A review of the status and stock structure of beluga whales, Delphinapterus leucas.

   5.3. Sample size and power

B. L. Taylor. Confidence in our results: sample size and power in stock definition and identifying individuals to stock.

   5.4. Individual identification as alternative to stock identification

P. J. Palsbøll. Population assignment based upon multi-locus data.

6. Market Surveys and Collection
   6.1. Past collection methods: sample documentation and preservation, and survey strategies: random sampling, targeted sampling

N. Funahashi. Sample collection methods for Japanese market surveys by IFAW, Addenda: Sample collection methods for South Korean market surveys by IFAW.

L. Grohmann, I. Bokermann. Greenpeace Germany Survey (1997).

M. J. Phipps. Past collection methods: Sample documentation and preservation and sampling strategies: TRAFFIC surveys.

   6.2. Detecting a minimum specified proportion (or "threshold") of a species in the market and detecting trends in market proportions

R. Leaper and N. Funahashi. A note on the data requirements and possible sampling design in order to determine the proportions of different product types on the market.

   6.3. Meta-analysis of past surveys

J. G. Cooke, R. Leaper, and G. M. Lento. Analysis of species composition of samples of edible whale products collected in Japanese markets, 1993-99, with some suggestions for future analysis methods.

7. Reference Samples and Archives
   7.1. Review of status of reference samples, including scientific access

K. M. Robertson and S. J. Chivers. Current status of the molecular genetics tissue archive at the Southwest Fisheries Science Center.

T. K. Yamada. Stranding network activities and their results, with brief comments on DNA sampling in Japan.

M. L. Dalebout. The New Zealand Stranded Cetacean Tissue Archive and DNA database.

G. M. Lento and C. S. Baker. Witness for the Whales Website.

A. K. Daníelsdóttir and G.A. Víkingsson. Availability of genetic samples of cetaceans in Iceland.

M. D. Santos. Management of regulated aquatic species in the Philippines: How DNA forensics could help?

P. J. Palsbøll. Palsbøll's sample database and completed analyses.

7.2. Procurement of, archiving of, and access to current and future genetic information

8. Other Business
   8.1. Recommendations
   8.2. Other business
   8.3. Adoption of draft report
   8.4. Close of the workshop

Appendix 4. Species identification within the problematic delphinids: *Stenella*, *Tursiops*, and *Delphinus*.

Dizon described a case study in species identification of ten sequences sent to the SWFSC by Cipriano which allowed a comparison of the NEM (near exact match) approach and a phylogenetic approach. The phylogenetic approach used neighbor-joining trees and tested relationships with bootstrap analyses. The dolphin taxa considered had high genetic diversity, were not monophyletic, and had low bootstrap support. Species identity was assigned to the unknown individual according to the species of the closest individual within the tree. Because there were no cases in which two known individuals were the same distance from the unknown, all ten samples were assigned to a species using this method. It has been suggested that the strength of the bootstrap value can be used to decide when a species identification is valid. Only one of the samples had a bootstrap value over 70%.

To check the robustness of this method to the size of the reference database, reference sequences were removed at random and the assignment process repeated. Four of the ten test animals were subsequently assigned to different species than that indicated using the full reference database.

The NEM approach used two types of data, the number of mismatches for the most closely related individuals to the unknown sample and the critical NEM, defined as one less mismatch than the number of mismatches known to occur between two reference sequences from the two plausible species under consideration. This critical NEM differed between species pairs. Of the 10 unknown samples, 4 had a number of mismatches to the closest reference sequence that was below the critical NEM; hence they were assigned to species with certainty. It was recognized that the uncertainty associated with this technique would be reduced as the reference database became more representative of the species.

Thus, the two approaches are both sensitive to the size and extent of the reference database. In some respects, the approaches are similar in that the closest relative found using the phylogenetic approach matched the smallest number of mismatches in nine of the ten samples. Use of the bootstrap value as a measure of reliability of the species identification could be overly conservative because fairly low values were seen even for test sequences that were identical or that differed by only one base-pair from reference sequences.

Appendix 5. A sample GenBank submission for an mtDNA control region sequence for an Atlantic white-sided dolphin (*Lagenorhynchus acutus*).

SAMPLE GenBank Submission

```
LOCUS             AF113486    451bp DNA   MAM   28-JUN-1999
DEFINITION        Lagenorhynchus acutus isolate Lacu.930C mitochondrial control
                  region, partial sequence.
ACCESSION         AF113486
KEYWORDS          .
SOURCE            Lagenorhynchus acutus.
  ORGANISM        Mitochondrion Lagenorhynchus acutus
                  Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Mammalia;
                  Eutheria; Cetartiodactyla; Cetacea; Odontoceti; Delphinidae;
                  Lagenorhynchus.
REFERENCE         1  (bases 1 to 451)
  AUTHORS         Cipriano,F.
  TITLE           Antitropical distributions and speciation in dolphins of the
                  genus Lagenorhynchus: a preliminary analysis
  JOURNAL         (in) Dizon,A.E., Chivers,S.J. and Perrin,W.F. (Eds.);
                  MOLECULAR GENETICS OF MARINE MAMMALS: 388; The Society for
                  Marine Mammalogy, P.O. Box 368, Lawrence, KS, USA (1997)
REFERENCE         2  (bases 1 to 451)
  AUTHORS         Cipriano,F.
  TITLE           Direct Submission
  JOURNAL         Submitted (15-DEC-1998) Center for Conservation and
                  Evolutionary Genetics, Harvard University, 16 Divinity Avenue,
                  Cambridge, MA  02138, USA
FEATURES          Location/Qualifiers
      source            1..451
                        /organism="Lagenorhynchus acutus"
                        /mitochondrion
                        /isolate="Lacu.930C"
                        /db_xref="taxon:90246"
                        /country="Canada:Newfoundland"
      misc_feature      <1..451
                        /note="control region"
BASE COUNT  140a  95c   55g   161t
ORIGIN
  1 gaacaagctt attgtataat taccacaaca ccacagtact atgtcagtat taaaaataat
 61 ttgttccaaa aaacatttat tatatacatc acatacatac atatacatgt caatatttag
121 tccttttca taaatattta tatgtacatg ctatgtatta ttgtgcattc atttattttc
181 catacgataa gttaaagctc gtattaatta tcattaattt tacatattac ataatttgca
241 tgctcttaca tattatatat cctctaacaa ttttatttcc attatatcct atggtcgctc
301 cattagatca cgagcttaat caccatgccg cgtgaaacca gcaacccgct cggcagggat
361 ccctcttctc gcaccgggcc catactcgtg ggggtagcta acagtgatct ttataagaca
421 tctggttctt acttcaggac cattttaact t//
```

Appendix 6. Summary analysis of species composition in Japanese market sample collections, 1993-99 (J. G. Cooke, R. Leaper and G. M. Lento).

The species composition from the collections reported to date are given in Table A1. These data are the same as those included in Table 1 but tabulated by collection year. The collection teams were divided into five groups:

- J—collections by the Fisheries Agency, Government of Japan
- L—collections organized by the Baker *et al.* group at the University of Auckland
- T—collections organized by TRAFFIC Japan
- G—collections organized by Greenpeace, Germany
- C—collections organized by Cipriano *et al.* at Harvard University

Because of the small numbers encountered for many species, species were combined into four groups:

- Nminke—orthern Hemisphere minke whales
- Sminke—Southern Hemisphere minke whales
- Baleen—Large baleen whales
- Odontocetes—All toothed whales

Non-cetacean samples from merchandise displayed as cetacean products were ignored. A generalized linear model was fitted to the combined frequency table using the following factors:

- SAMPLE—(dummy parameter for sample size, equivalent to conditioning on sample size by team by year)
- SPECIES
- SPECIES.T—(T = time [year] as a quantitative [trend] variable) (optional)
- SPECIES.TEAM—(optional)

The counts were treated as Poisson-distributed random variables, which is equivalent to treating the species composition within each collection as multinomially distributed. The log link was used. Significance levels for the optional terms were calculated in the usual way from the deviance reduction, but in view of the small numbers in some cells, Monte-Carlo significance levels were also estimated by simulation (1000 trials) as a cross-check.

The results of the analysis of deviance are given in Appendix 6, Table A2. Both the nominal and the simulated significance levels are given. The trend in species composition over time is highly significant ($P < 0.001$). No significant difference in species composition between collection teams is found ($P \approx 0.2$).

Table A1. Species breakdown of Japanese market sample collections used for analyses of collection teams and trends.

	Collection year											
Species	1993	1995	1995	1995	1996	1996	1997	1997	1998	1999	1999	Total
N. minke	3	5	2	12	9	2	10	8	19	21	29	120
S. minke	22	63	31	112	46	2	18	22	51	35	56	458
Dwarf minke	0	0	0	1	0	0	0	0	0	0	0	1
Bryde's	1	2	1	0	0	0	1	0	0	0	1	6
Pygmy Bryde's	0	0	0	0	0	0	0	0	0	0	0	0
Sei	0	0	0	0	0	0	1	0	3	1	0	5
Humpback	1	0	0	0	0	0	1	0	0	0	2	4
Fin	7	6	2	15	2	0	6	3	2	1	0	44
Blue	1	1	0	0	0	0	0	0	0	0	0	2
Sperm	0	0	0	2	0	0	0	0	0	1	2	5
Pygmy sperm	0	0	0	0	0	0	0	0	1	0	0	1
Other beaked	0	0	0	3	0	1	0	1	0	0	0	5
Baird's beaked	0	11	2	14	5	0	4	1	6	6	7	56
Cuvier's beaked	1	2	1	0	1	0	0	0	0	0	0	5
Porpoise	0	2	6	0	6	1	0	1	1	1	1	19
Killer whale	0	0	0	0	0	0	1	0	0	0	0	1
Dolphins	5	14	4	1	18	1	3	2	13	9	15	85
Cetacean Total	41	106	49	160	87	7	45	38	96	75	113	817
Artiodactyl										1		1
Sheep		2										2
Horse									2			2
Total	41	108	49	160	87	7	45	38	98	76	113	822
Team	L	L	T	J	C	L	L	G	L	L	C	
Reference	1	3	12	7	5	9	9	8	10	4	6	

Table A2. Analysis of deviance Species breakdown of Japanese market sample collections.

Model	Deviance	d.f.	*P* (calc)	*P* (simulated)
Sample + Species	106.58	30		
Sample + Species	50.78	27	0.0001	<0.001
Sample + Species + Species.T + Species.Team	24.62	15	0.298	0.169

Appendix 7. A glossary of molecular genetic, evolutionary, statistical, etc. terminology.

Alignment—Juxtaposition of amino acids or nucleotides in homologous molecules that are assumed to be positional homologs.

Amplify—To increase the amount of DNA to levels useful for analysis, *e.g.,* by the polymerase chain reaction (PCR).

Annealing—Pairing of complementary strands of DNA to form a double helix.

Bootstrap—A statistical method based on repeated random sampling with replacement from an original sample to provide a collection of new estimates of some parameter, from which confidence limits can be calculated.

Cladistic—A system of phylogenetic reconstruction and classification in which the only groups formally recognized are monophyletic groups (clades) and which hypotheses of relationship are based strictly on genealogy.

Control region—A noncoding portion of the mtDNA molecule functional in replication.

Cytochrome *b*—A mitochondrial gene involved in respiration, used extensively in exploring phylogenetic relationships at and above the species level.

Electrophoretogram—Trace produced by separation of molecules in an electric field.

Haplotype—Particular combination of alleles in a defined region of some chromosome or the mtDNA molecule, or particular combinations of sequence fragments (RFLPs).

Heteroplasmy—Variation in genotype within the same individual.

Homoplasy—The repeated appearance of similar features in two or more unrelated (i.e., not directly descendant) taxa.

Jackknife—A statistical method of numerical resampling based on n samples of size n-1 used to calculate the variance of an estimate from an original sample of size n.

Ligation—Formation of a bond to link two adjacent bases separated by a nick in one strand of a double helix of DNA.

Locus—The position on a chromosome/mtDNA molecule at which the gene for a particular trait or an intron resides.

Marker—Any allele of interest in an experiment or analysis; proxy for a targeted gene or characteristic.

Maximum likelihood—Statistical procedure for estimating population parameters (of all possible values) most likely to yield the samples observed.

Microsatellites—Non-coding short tandem repeats in the nuclear genome; those having variable number of repeats (alleles) used in population analysis.

Mitochondrial DNA (mtDNA)—DNA contained in the mitochondrian, in a single circular molecule; maternally transmitted.

Neighbor-joining—Tree-building algorithm based on relationship by similarity.

Node—The graphic representation in a phylogram of an extant or ancestral taxon/individual.

Nuclear translocation of mtDNA (NuMt)—Insertion of a fragment of mtDNA in the nuclear genome; acts as molecular fossil that may lead to errors in mtDNA sequencing.

Nucleotide—Any of the basic building blocks of nucleic acids.

Oligonucleotide—A short chain of nucleotides, often produced in the laboratory.

Operon—Unit of gene expression and regulation.

Paraphyletic—In phylogeny, taxon or entity not containing all the descendants of a single common ancestor, as opposed to monophyletic.

Parsimony—A method of phylogenetic tree inference based on the principle of minimizing the amount of evolutionary change needed to explain the data.

Phylogenetic—Based on inferred genealogical relationships among entities.

Polymerase chain reaction (PCR)—A biochemical procedure used in amplifying DNA to levels useful for analysis.

Polymorphic—A character (gene) with two or more distinct states (alleles).

Power—In statistics, the ability of an analysis to correctly reject the null hypothesis, *i.e.,* to not miss a real effect.

Primer—A short nucleic acid sequence paired with one strand of DNA and at which a polymerase initiates synthesis of a DNA chain.

Pseudogene—A gene sequence closely homologous to a functional gene but disabled by mutations that prevent it from being expressed.

Reference sequence—A DNA sequence of known identity to which a sample of unknown identity is compared.

Restriction endonuclease—An enzyme used to break up a nucleic acid sequence into segments at specific sites.

Stop codon—Sequence of nucleotides that signals the end of transcription.

Substitution—Replacement of one nucleotide by another in a nucleic acid sequence.

Synonymous—A substitution that does not change the coding of a three-nucleotide sequence for a particular amino acid.

Test sequence—A DNA sequence of unknown identity to be compared to a reference sequence.

Transition—A mutation in which one pyrimidine is substituted by another or one purine substituted by another.

Transversion—A mutation in which a pyrimidine is substituted by a purine or vice versa.

[This page intentionally left blank]

RECENT TECHNICAL MEMORANDUMS

Copies of this and other NOAA Technical Memorandums are available from the National Technical Information Service, 5285 Port Royal Road, Springfield, VA 22167. Paper copies vary in price. Microfiche copies cost $9.00. Recent issues of NOAA Technical Memorandums from the NMFS Southwest Fisheries Science Center are listed below:

NOAA-TM-NMFS-SWFSC-

276 Ichthyoplankton and station data for California Cooperative Oceanic Fisheries Investigations survey cruises in 1995.
E.M. SANDKNOP, R.L. CHARTER, and H.G. MOSER
(September 1999)

277 Ichthyoplankton and station data for California Cooperative Oceanic Fisheries Investigations survey cruises in 1996.
W. WATSON, R.L. CHARTER, AND H.G. MOSER
(September 1999)

278 Ichthyoplankton and station data for California Cooperative Oceanic Fisheries Investigations survey cruises in 1997.
D.A. AMBROSE, R.L. CHARTER, AND H.G. MOSER
(September 1999)

279 Ichthyoplankton and station data for California Cooperative Oceanic Fisheries Investigations survey cruises in 1998.
S.R. CHARTER, R.L. CHARTER, AND H.G. MOSER
(September 1999)

280 Hawaiian Monk Seal epidemiology plan: Health assessment and disease status studies.
A.A. AGUIRRE, J.S. REIF, and G.A. ANTONELIS
(October 1999)

281 The Kewalo Research Facility - Leading the way for more than 40 years.
R.W. BRILL
(October 1999)

282 282 "U.S. Pacific marine mammal stock assessment: 1999"
K.A. FORNEY, M.M. MUTO, and J. BAKER
(October 1999)

283 283 Marine mammal data collected during a survey in the eastern tropical Pacific Ocean aboard the NOAA ships *McArthur* and *David Starr Jordan* and the UNOLS ship *Endeavor* July 31-December 9, 1998.
D. KINZEY, T. GERRODETTE, J. BARLOW, A. DIZON, W. PERRYMAN, P. OLSON, and A. VON SAUNDER
(November 1999)

284 Length-weight interrelationships for swordfish, *Xiphias gladius* L., caught in the central north Pacific.
J.H. UCHIYAMA, E.E. DeMARTINI, and H.A. WILLIAMS
(December 1999)

285 Continuous high resolution shore station temperature and salinity data from Granite Canyon, California.
J.G. NORTON, C.S. MOORE, F.B. SCHWING, D. HUSBY, D. BALTZ, H. PARKER-HALL, D. Ven TRESCA, and D.M. FERNANDEZ
(December 1999)